



**University of
Zurich^{UZH}**

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2013

Phylogeny and expression divergence of metabotropic glutamate receptor genes in the brain of zebrafish (*Danio rerio*)

Haug, Marion F ; Gesemann, Matthias ; Mueller, Thomas ; Neuhauss, Stephan C F

Abstract: Glutamate, the most abundant excitatory neurotransmitter of the central nervous system, modulates synaptic transmission and neuronal excitability via metabotropic glutamate receptors (mGluRs). These receptors are essential components for diverse cognitive functions and they represent potential drug targets for the treatment of a number of neurological and psychiatric disorders. Here, we describe the phylogenetic relation and mRNA distribution of zebrafish mGluRs. In comparison to the eight mglurs present in the mammalian genome, we identified 13 different mglur genes in the zebrafish genome. In situ hybridization experiments in zebrafish revealed widespread expression patterns for the different mglurs in the central nervous system, implicating their significance in diverse neuronal functions. Prominent mglur expression is found in the olfactory bulb, the optic tectum, the hypothalamus, the cerebellum, and the retina. We show that expression pattern of paralogs generated by the teleost specific whole genome duplication is overlapping in some brain regions but complementary in others, suggesting sub- and/or neofunctionalization in the latter. Group I mglurs are similarly expressed in brain areas of both larval and adult zebrafish, suggesting that their functions are comparable during these stages.

DOI: <https://doi.org/10.1002/cne.23240>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-66344>

Journal Article

Originally published at:

Haug, Marion F; Gesemann, Matthias; Mueller, Thomas; Neuhauss, Stephan C F (2013). Phylogeny and expression divergence of metabotropic glutamate receptor genes in the brain of zebrafish (*Danio rerio*). *Journal of Comparative Neurology*, 521(7):1533-1560.

DOI: <https://doi.org/10.1002/cne.23240>

**Phylogeny and Expression Divergence of Metabotropic
Glutamate Receptor Genes in the Brain of Zebrafish (*Danio
rerio*)**

Journal:	<i>The Journal of Comparative Neurology</i>
Manuscript ID:	JCN-11-0344.R5
Wiley - Manuscript type:	Research Article
Keywords:	metabotropic glutamate receptor, zebrafish, brain, retina, central nervous system

SCHOLARONE™
Manuscripts

Phylogeny and Expression Divergence of Metabotropic Glutamate Receptor Genes in the Brain of Zebrafish (*Danio rerio*)

mGluRs in the Zebrafish Brain

Marion F. Haug¹, Matthias Gesemann¹, Thomas Mueller², Stephan C. F. Neuhauss^{1§}

¹Institute of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

²Institute of Biology I, Faculty of Biology, Department of Developmental Biology, University of Freiburg, Hauptgasse 1, D-79104 Freiburg, Germany

§Corresponding author

Stephan C.F. Neuhauss
Institute of Molecular Life Sciences
Winterthurerstrasse 190
8057 Zürich
Phone: ++41 (0)44 635 60 40
Fax: ++41 (0)44 635 68 97
stephan.neuhauss@imls.uzh.ch

Keywords: mGluR, central nervous system, retina

Abstract

Glutamate, the most abundant excitatory neurotransmitter of the central nervous system, modulates synaptic transmission and neuronal excitability via metabotropic glutamate receptors (mGluRs). These receptors are essential components for diverse cognitive functions and they represent potential drug targets for the treatment of a number of neurological and psychiatric disorders.

Here, we describe the phylogenetic relation and mRNA distribution of zebrafish mGluRs. In comparison to the eight *mglurs* present in the mammalian genome, we identified 13 different *mglur* genes in the zebrafish genome. *In situ* hybridization experiments in zebrafish revealed widespread expression patterns for the different *mglurs* in the central nervous system, implicating their significance in diverse neuronal functions. Prominent *mglur* expression is found in the olfactory bulb, the optic tectum, the hypothalamus, the cerebellum, and the retina. We show that expression pattern of paralogs generated by the teleost specific whole genome duplication is overlapping in some brain regions but complementary in others, suggesting sub- and/or neofunctionalization in the latter. Group I *mglurs* are similarly expressed in brain areas of both larval and adult zebrafish, suggesting that their functions are comparable during these stages.

Introduction

In recent years the zebrafish *Danio rerio* has emerged as one of the favorite model organisms for genetic studies. Particularly well studied are genes involved in nervous system development and function (e.g. Schweitzer and Driever, 2009; Lillesaar, 2011). However, despite the fact that glutamate is the main excitatory neurotransmitter of the vertebrate nervous system, little is known about glutamatergic systems in zebrafish or other teleosts.

In general, glutamate receptors fall into three broad classes: the ionotropic NMDA and AMPA/kainate receptors, the excitatory amino acid transporters (EAATs) and the mGluRs. mGluRs are seven-transmembrane proteins with an intracellular G-protein coupled signal transduction pathway, which activates a second messenger cascade upon glutamate binding (Nakanishi and Masu, 1994). In mammals, the mGluR family consists of eight different subtypes that can be classified into three distinct groups (I-III) based on their structural homology, pharmacological properties and second messenger cascade. While receptor subtypes -1 and -5 comprise the group I, mGluR2 and -3 are members of group II, and mGluR4, -6, -7 and -8 are constituting the group III. Group I members are positively coupled to the phosphoinositol transduction pathway whereas group II and group III mGluRs are negatively linked to adenylyl cyclase and thus downregulate cyclic nucleotide synthesis. Group III mGluRs can be distinguished from group II mGluRs due to their distinctive sensitivity for the agonist L-2-amino-4-phosphonobutyrate (APB or L-AP4) (Pin and Duvoisin, 1995; Rosemond et al., 2004).

By modulating signal transduction at pre- and postsynaptic sites, glutamate acting via mGluRs plays a crucial role in neuronal synaptic transmission. Consequently, mGluRs can be found in diverse neuronal cell types of the central and peripheral nervous system and they have been considered potential targets for therapeutic approaches in various neurodegenerative

disorders (Byrnes et al., 2009; reviewed in Knackstedt and Kalivas, 2009; Luscher and Huber, 2010; Niswender and Conn, 2010). The functions of group I mGluRs have been especially well-studied as they are expressed in many excitatory synapses (reviewed in Ferraguti and Shigemoto, 2006). Group I mGluRs are implicated in long term potentiation (LTP) influencing plasticity of behavior, learning and memory. Moreover, mGluR-induced LTP might be linked to aspects of mental retardation as well as Parkinson's and Alzheimer's disease (reviewed in Luscher and Huber, 2010). In this study, we cloned and phylogenetically characterized all members of the *mglur* family in the zebrafish *Danio rerio*. Similar to mammals, the zebrafish mGluR subtypes fall into the three distinct groups, however, *mglur1*, -2, -5, -6, and -8 are present as two paralogs. Using *in situ* hybridization, we analyzed their expression patterns during development and found a unique pattern for all *mglurs* in larval zebrafish. Paralogous genes show overlapping but also mutually exclusive expression domains, suggesting potential sub- or neofunctionalization between duplicated genes (Force et al., 1999). In addition, we studied gene expression patterns of group I *mglurs*, namely *mglur1a* and *-1b*, as well as *mglur5a* and *-5b*, on brain sections of adult zebrafish in order to compare expression patterns between larval and adult stages. These transcript patterns, here exemplified for the cerebellum and the hypothalamus, show similarities between embryonic and adult zebrafish indicating conserved functions throughout the stages.

Materials and Methods

Fish maintenance and breeding

Fish were kept under a 14h/10h light/dark cycle and bred as previously described (Mullins et al., 1994). The wild-type strain used for all studies was WIK. Embryos were raised at 28 °C in E3 medium and staged according to development in days post fertilization (dpf). Experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the local authorities (Veterinäramt Zürich TV4206).

Annotation of *mglur* cDNAs

As gene predictions within GenBank are produced by automated processes which have been shown to contain numerous errors, mGluR cDNA sequences used in this study were manually annotated. Sequences were identified and annotated using combined information from expressed sequence tags and genome databases (GeneBank, <http://www.ncbi.nlm.nih.gov>; Ensembl, <http://www.ensembl.org/index.html>). Human and mouse sequences were used as initial query (for more details on sequence annotation see Gesemann et al., 2010).

Phylogenetic analysis

The phylogenetic analysis was performed on the Phylogeny.fr platform (<http://www.phylogeny.fr>) comprising the following steps (Dereeper et al., 2008). Sequences were aligned using MUSCLE (v3.7) (Edgar, 2004) configured for highest accuracy (MUSCLE with default settings). Sequences length varied between 727 and 1199 amino acids. After alignment, ambiguous regions (i.e. containing gaps and/or are poorly aligned) were removed

using Gblocks (v0.91b) (Castresana, 2000). The following parameters were implemented. The minimum length of a block after gap cleaning was set to 5; positions with a gap in less than 50% of the sequences were selected in the final alignment if they were within an appropriate block; all segments with contiguous nonconserved positions bigger than 8 were rejected; minimum number of sequences for a flank position were 55%. After curation 703 amino acids were chosen for further analysis. The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0 aLRT) (Guindon and Gascuel, 2003). The default substitution model was selected assuming an estimated proportion of invariant sites (of 0.000) and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data ($\gamma = 0.728$). Reliability for internal branch was assessed using the aLRT test (Anisimova and Gascuel, 2006). Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3) and the svg file imported into CorelDraw (version x4; Corel Corporation Ottawa, Canada) for final editing.

Cloning of *mglur* full-length cDNAs

Using the QIAshredder and the RNeasy kit (Qiagen, Hombrechtikon, Switzerland), the total mRNA of approximately 80 7 day old wild-type zebrafish heads was isolated and reverse transcribed using oligo-dT primers (First Strand Kit, Stratagene, La Jolla, CA, USA). For Polymerase Chain Reaction (PCR) Taq polymerase (Taq Gold; Applied Biosystems, Switzerland) and sequence-specific oligonucleotide primers were used (Table 1). Amplified DNA pieces were subcloned into the TOPO pCRII vector (TA Cloning Kit Dual Promoter, Invitrogen, Basel, Switzerland) and subsequently sequenced.

Whole mount *in situ* hybridization

In vitro transcription of DNA probes was performed using the Roche DIG-RNA Labeling Kit (Roche Diagnostics, Rotkreuz, Switzerland). Probes longer than 1000 bp were hydrolyzed. To suppress pigmentation, embryos were treated with 3 μ M PTU (1-phenyl-2-thiourea, Sigma-Aldrich, St. Louis, MO, USA). Zebrafish larvae were collected at 3 or 5 dpf, anesthetized on ice, and immediately fixed in 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS, freshly prepared, pH 7.4) over night (ON) at 4 °C. Fixed tissue was washed twice for 5 min with PBT (PBS containing 0.1% Tween 20, Sigma-Aldrich) and dehydrated in a graded series of methanol/PBT mixtures each for 5 min at room temperature (RT). Larvae were stored at -20 °C in 100% methanol until required. Whole mount *in situ* hybridization was performed according to Thisse and Thisse (Thisse and Thisse, 2008) with slight adaptations of the protocol: permeabilization by proteinase K (10 μ g/ml) was accomplished for 60 min for 3 day old fish and for 75 min for 5 day old fish. From day two on TNT (100 mM Tris HCl pH 7.5, 150 mM NaCl, 0.5% Tween 20) was used for all washing steps instead of PBT. AP-conjugated anti-DIG antibody (Roche) was diluted 1:5'000 in blocking solution (Roche) in TNT. After the staining was stopped, larvae were postfixed in 4% PFA in PBS ON at 4 °C. The next day, stained larvae were washed twice for 5 min in PBT and placed in successive dilutions of PBT/methanol and methanol/glycerol each for 5 min at RT to the final step of 100% glycerol (Sigma-Aldrich). For obtaining optimal pictures, larvae were mounted on an adapted glass slide in 100% glycerol (Sigma-Aldrich) and the DIC modus of a light microscope (Olympus BX61) and a color camera (ColorView IIIu, Soft Imaging System, Olympus) were used. Representative whole mount stained larvae were placed in 30% sucrose ON at 4 °C and embedded in cryomatrix (Tissue Tek O.C.T., Sakura, Zoeterwonde, NL) in an aluminum mold. 12 μ m thick transverse sections were cut using a Microm microtome (HM 550) collected on glass slides and cover-slipped with

Kaiser's glycerol gelatine (Merck KGaA, Darmstadt, Germany). Images were taken in the bright field modus of a light microscope (Olympus BX61). They were adjusted for brightness and contrast and arranged using Adobe Photoshop and Adobe Illustrator CS5.

***In situ* hybridization on sections**

Adult zebrafish were euthanized using tricaine (MS-222, Sigma-Aldrich) and iced water. The head was cut, briefly washed in PBS and fixed in 4% PFA ON at 4 °C. After washing the tissue twice with PBT for 5 min at RT, it was placed in 30% sucrose ON and treated similar to the whole mount stained larvae. Eventually, 16 µm thick transverse and sagittal sections were cut and mounted on glass slides. *In situ* hybridization was performed as described for larval fish, but proteinase K permeabilization time was reduced to 2.5 min and PBT was used for the washing steps and to dissolve the blocking reagent. The staining was stopped by a brief washing step with PBS pH 5.5 followed by two washing steps with PBS pH 7.4. Before the cover-slip was mounted, the stained tissue was postfixed in 4% PFA at RT for 1 h and washed twice with PBS pH 7.4. Images were taken with the DIC modus of a light microscope (Olympus BX61) and were processed and arranged using Adobe Photoshop and Adobe Illustrator CS5.

Results

mGluRs in the zebrafish *Danio rerio*

The family of metabotropic glutamate receptors in mammals consists of eight different members which are categorized into three subgroups according to their sequence homology, pharmacology, and associated signal transduction pathways (Pin and Duvoisin, 1995). Based on sequence similarity, we annotated and cloned 13 zebrafish mGluR family members. Except for some single nucleotide polymorphisms, sequencing of the amplified cDNA fragments revealed no significant alterations from our annotated sequences. We distinguished three subgroups of zebrafish mGluRs which correspond to their mammalian counterparts (Fig. 1). While we found no evidence for a second zebrafish paralog of *mglur3*, -4, and -7, we detected two paralogs for *mglur1*, -2, -5, -6, and -8. The location of duplicated *mglurs* on different chromosomes supports the notion that they originated from the teleost specific whole genome duplication rather from tandem duplication (Meyer and van de Peer, 2005; Ohno, 1999; Postlethwait, 2007).

Distinct expression patterns for *mglurs* in the larval and adult zebrafish nervous system

To determine the expression pattern of members of the metabotropic glutamate receptor family, we performed *in situ* hybridization experiments on five-day-old zebrafish. An overview of *mglur* expressions in larval zebrafish is shown in Table 2. We generally followed the nomenclature of the zebrafish brain atlas of larval (Mueller and Wullmann, 2005) and adult (Wullmann et al., 1996) zebrafish and followed some modifications suggested by other authors (Bae et al., 2009; Wullmann and Mueller, 2004a; Mueller et al., 2006).

Transcripts for both *mglur1* paralogs are located in the region of the cerebellar plate (Figs. 2+3). However, the expression domains of *mglur1a* transcripts are broader and the

expression levels appear to be higher compared to its paralog *mglur1b*. In the adult cerebellum, *mglur1a* is exclusively expressed in the Purkinje cells (PCL; Fig. 4A,H-K). In contrast, *mglur1b* is located in Purkinje cells but additionally in the granule cell layer of the corpus cerebelli and in the lobus caudalis cerebelli (GCL, LCa; Fig. 4L,R-T). Expression of *mglur1b* in the eminentia granularis is seen throughout development (EG; Fig. 3) and persists into adulthood (Fig. 4S). While *mglur1a* is strongly expressed in the olfactory bulb of the mature brain (OB; Fig. 4A), expression in the larval telencephalon was found to be in the subpallial region (Sd; Fig. 2K) although at 3 dpf expression in the olfactory bulb cannot be excluded (OB, Sd; Fig. 2B,F). The faint *mglur1a* expression in diencephalic regions of zebrafish larvae (Fig. 2E,J,L-M) stands in contrast to the strong labeling of these structures in the adult fish (Fig. 4A,F-K). *mglur1b* is largely expressed in the same diencephalic regions but shows an additional staining of the epiphysis at 5 dpf (E; Fig. 3G,I,J). It is furthermore expressed in the lateral tectal proliferation zone as well as in the optic tectum of three- and five-day-old larval zebrafish (l, TeO; Fig. 3A,D,G,H,K-N). The optic tectum of adult fish shows *mglur1b* expression domains comparable to larval ones (TeO; Fig. 4L,P-R). No *mglur1b* labeling, however, was found in telencephalic and medial to ventral diencephalic regions at larval stages in contrast to the adult CNS (Fig. 4L,O-S). Moreover, cells of the medial octavolateralis nucleus display a very prominent *mglur1b* labeling (MON; Fig. 4L,T).

In larval fish *mglur5* paralogs are strongly expressed in the pallium (P), in hypothalamic parts of the brain (Hi, Hr, DIL, TLA), and weakly expressed in the cerebellar plate (CeP) and the nucleus interpeduncularis (Nln; all in Figs. 5+6). Paralog specific staining for *mglur5a* can be found in a ventral part of the hindbrain, most likely in the inferior olive (IO, Fig. 5B,F,H,J,Q). The localization of *mglur5a* riboprobes in adult tissue highly resembles the larval situation. In adult fish, a prominent labeling of dorsal telencephalic regions (Dd, Dm, Dl, Dp; Fig. 7A,D-F),

hypothalamic structures (Hd, Hv, LH, CIL, DIL, TLa; Fig. 7A,H-K) and the nucleus interpeduncularis (Fig. 7K) was found. In lateral sections even cell bodies of the inferior olive are *mglur5a* positive (data not shown). The *mglur5a* probe specifically labels Purkinje cells (PCL; Fig. 7A), but we interpreted the weak labeling of the cerebellar granular cell layer as unspecific, because it was not consistent on all sections. In addition, the olfactory bulb (OB; Fig. 7A) and the caudal part of the periventricular hypothalamus (Hc; Fig. 7A,J) is stained in adult tissue. In contrast to this, *mglur5b* is broadly expressed in various parts of the diencephalon (DT, EmT, M2; Fig. 6), and the medulla oblongata (MO; Fig. 6) in three- and five-day-old fish. In the adult CNS, *mglur5b* expression is restricted to frontal brain regions such as the olfactory bulb and the dorsal and ventral telencephalic areas (OB, Vc, Vd, VI, Vv; Fig. 7L,O-Q). The periventricular gray zone of the optic tectum (PGZ; Fig. 7S-W) and some diencephalic structures such as the preglomerular nucleus (PGL, PGm; Fig. 7T), and hypothalamic nuclei (Hc, Hd, Hv, LH, TLa, DIL; Fig. 7L,T-W) are strongly labeled.

The group II mGluRs in zebrafish consist of two mGluR2 paralogs and mGluR3. The *mglur2a* riboprobe labels cells in the olfactory bulb (OB), migrated neurons of the eminentia thalami (M3), as well as lateral parts of the cerebellar plate (CeP), and the trigeminal ganglion (TG). In addition, a weak staining in several parts of the midbrain (DT, VT, PTv) and in the caudal tegmentum/rostral medulla oblongata (T, MO; all in Fig. 8) is visible. Sections show additional *mglur2a*-positive cells in the eminentia granularis (EG; Fig. 8Q). Similar to *mglur2a*, *mglur2b* is expressed in the olfactory bulb, however, expression of this paralog is restricted to the medial part of the olfactory bulb (OB; Fig. 9). Besides that the paralogous genes do not share other expression domains. Additional *mglur2b*-specific expression is found in the pallium (P), in proliferative cells around the ventricular zones (TVe, TeVe, RVe), the epiphysis (E), the intermediate and caudal hypothalamus (Hi, Hc), the reticular formation (RF), and in a nucleus in

the lateral part of medulla oblongata (MO; all in Fig. 9). In three-day-old larvae *mglur2b* is also expressed in a structure which might be the Mauthner cells (MC; Fig. 9C,F) and in brain sections at 5 dpf we see a staining in the region of the griseum tectale (GT; Fig. 9M). To conclude, *mglur2b* is expressed in medial part of the olfactory bulb (Fig. 9), whereas *mglur2a* is expressed within the entire OB (Fig. 8). Otherwise, these two paralogous genes are expressed mutually exclusive in the brain of larval fish.

Riboprobes for *mglur3* stain the proliferative, periventricular zones of the larval zebrafish brain surrounding the telencephalic (TeV), the tectal (TeV), and the rhombencephalic ventricles (RVe; all in Fig. 10). The periventricular zone of the lateral ventricular recess of the hypothalamus is faintly stained (LVe; Fig. 10O). Apart from that, *mglur3* is also expressed in the pallium (P), the hypothalamus (Hi, Hc), the cerebellum (Va, CeP). Some weak *mglur3* expression is found in the habenula (Ha), the migrated part of the posterior tubercular area (M2), and the superior raphe (SR; all in Fig. 10).

Group III *mglur4* transcripts are located in multiple regions of the CNS. The telencephalic part shows a staining in the olfactory bulb (OB), the dorsal division of the subpallium (Sd), and the preoptic region (Po; all in Fig. 11). Besides intense staining of the optic tectum (TeO) and the tegmentum (T), diencephalic regions (Ha, PT, Hr, Hi, TLa; all in Fig. 11) are also stained with the *mglur4* riboprobe. The hindbrain is *mglur4*-positive in the lateral cerebellar plate (CeP), in a lateral nucleus, and a broad part of the medulla oblongata (MO; all Fig. 11). In addition, *mglur4* is strongly expressed in diverse cranial ganglia (TG, ALLG, FG, GG, VG, PLLG; all Fig. 11) at 3 and 5 dpf while expression in motor neurons (mn; Fig. 11A,E) is only found at 3 dpf.

Expression of both *mglur6* paralogs is limited in the CNS. The *mglur6a* probe strongly labels the habenula (Ha), the nucleus interpeduncularis (Nln), the lateral cerebellar plate (CeP), and the inferior olive (IO; all Fig. 12). In cross sections additional weak staining can be observed

in the subpallial region (Sd), in various parts of the diencephalon (between DT and PT, TeO, Hc) and in substructures of the medulla oblongata (MO; all Fig. 12). Besides staining in some scattered neurons of the olfactory bulb (OB) and the habenula (Ha), *mglur6b* reveals strong labeling in the rostral part of the hypothalamus (Hr; all Fig. 13). Both *mglur6* paralogs are highly expressed in inner retinal layers in both 3 and 5 day old larvae (GCL, INL; Figs. 12+13).

mglur7 transcripts are broadly expressed throughout the CNS in 3- and 5-day-old fish larvae. The telencephalon shows staining in the pallium (P) and the preoptic region (Po; both Fig. 14). Further expression is visible in diencephalic regions such as the eminentia thalami (EmT), the migrated area of the EmT (M3), the posterior tuberculus (M2), the optic tectum (TeO), the hypothalamus (Hr, TLa, DIL), and the nucleus of the inferior lobe (IO; all in Fig. 14). In addition, *mglur7* transcripts can also be seen in cerebellar regions (CeP), two cranial ganglia (TG, VG), the nucleus interpeduncularis (Nln), and in a lateral part of the medulla oblongata (MO; all in Fig. 14). Although staining in the habenula (Ha) is not seen in the cross section J of Figure 14, other sections reveal *mglur7* positive cells in this region (data not shown). In the retina *mglur7* is highly expressed in inner layers (GCL, INL; Fig. 14Q). At 3 dpf, motor neurons of the hindbrain reveal a faint labeling (mn; Fig. 14A) but apart from that *mglur7* expression in younger zebrafish larvae is identical to the one observed at 5 dpf.

mglur8a antisense RNA specifically labels the olfactory bulb (OB), the pallium (P, Sd), and the preoptic region of the forebrain (Po; all in Fig. 15). Transcripts are also found around migrated neurons of the eminentia thalami (M3), possibly in the ventral thalamus (VT?) and in the dorsal thalamus (DT), as well as in the optic tectum (TeO) the rostral part of the hypothalamus (Hr), and in the torus semicircularis (TS; all in Fig. 15). *mglur8a* expression in the hindbrain is confined to the cerebellar region (CeP) and parts of the medulla oblongata (MO; both in Fig. 15). While *mglur8a* is expressed in the proximal part of the inner nuclear layer (INL) and

in the ganglion cell layer (GCL; Fig. 15J8), its paralog is only found in the proximal INL (Fig. 16S). Overall, *mglur8b* shows a similar but weaker expression than *mglur8a* (Fig. 16). It is expressed in some additional structures such as the habenula (Ha), the posterior tuberculum (PT), the anterior tegmentum (T), and two cranial ganglia (OG, PLLG; all in Fig. 16).

At larval stages, besides *mglur2b* and *mglur5a*, all *mglurs* are expressed in the zebrafish retina. A closer analysis of *mglur5* paralogs in the adult retina reveals an expression of them in the inner nuclear layer (INL). In contrast to findings in mammals *mglurs* are located in either the inner nuclear layer (INL), or the ganglion cell layer (GCL), or in both, but never in photoreceptors.

Discussion

While the mammalian genome contains eight *mglur* genes (Nakanishi and Masu 1994), we found 13 mGluRs to be present in zebrafish. This increase in number can be readily explained by the whole genome duplication in teleosts (Meyer and van de Peer, 2005; Ohno, 1999; Postlethwait, 2007). The high amino acid sequence similarity as well as the segregation of the 13 zebrafish mGluRs into the corresponding subgroups suggests functional conservation of these receptors between zebrafish and mammals. Moreover, some of the *mglur* genes in zebrafish are known to be expressed in corresponding brain regions of mammals (reviewed in Ferraguti and Shigemoto, 2006), indicating that these genes are also similarly regulated.

In this study we determined the expression patterns of every single *mglur* in the zebrafish central nervous system (CNS). While expression of paralogous genes is overlapping in some brain regions, other areas are exclusively stained by the riboprobe of one paralog, suggesting evolutionary events such as sub- or neofunctionalization. Such events are best explained by the duplication-degeneration-complementation (DDC) model of Force and colleagues (Force et al., 1999). It states that accumulation of mutations in regulatory regions may lead to a split in gene function among gene paralogs (subfunctionalization) or even to the uptake of a novel, non-redundant function of either one paralog (neofunctionalization). In general, zebrafish *mglur* expression patterns often resemble the mammalian situation, which is also true for group I *mglurs* which were investigated in both adult as well as larval tissue. In the following paragraphs we will discuss how class I mGluRs expression patterns in the brain of zebrafish compare to orthologous gene expression patterns described for mammals. *mglurs* of other classes will be discussed in case they are prominently expressed in distinct brain regions.

Group I mGluRs

Group I *mglurs* are amongst the best studied members of the *mglur* family. Interestingly, our experiments indicated that the distribution of group I mGluRs as described in the rodent brain (reviewed in Ferraguti and Shigemoto, 2006) is strikingly similar to those of their orthologous genes in the zebrafish CNS. This suggests that these receptors have conserved functions between different vertebrates. In addition, group I *mglurs* are similarly expressed in both larval and adult zebrafish, suggesting that mGluR functions are established early during development. Early establishment may reflect the fact that neural circuits do not switch their receptor expression pattern once they have developed. The high resemblance between larval, adult as well as mammalian *mglur* transcript expression patterns speaks to the possibility to use zebrafish across different developmental stages to study mGluR function.

In rats, mGluR1 expression is strongest in the olfactory bulb, hippocampus, thalamus and cerebellar Purkinje cells, mGluR5 expression is present in many telencephalic and thalamic regions as well as in the superior colliculus (reviewed in Ferraguti and Shigemoto, 2006). In contrast to mammals, zebrafish possess two *mglur1* and *mglur5* paralogs each, so some of the functions may have been distributed onto only one paralogous gene. mGluR1 for example is involved in learning and memory in mammals (Aiba et al., 1994a; Gil-Sanz et al., 2008), a hypothesis that is supported by the expression of both mammalian *mglur1* splice variants in the hippocampus (Martin et al., 1992; Baude et al., 1993; Ferraguti et al., 1998, 2004). In zebrafish, only *mglur1a* but not *-1b* is expressed in the lateral dorsal telencephalic area, the regions that corresponds to the mammalian hippocampus (Wullimann and Mueller, 2004b; Northcutt, 2006). We therefore hypothesize that a possible mGluR1 involvement in learning and memory in this area is solely transferred onto the zebrafish mGluR1a.

Olfactory bulb

The OB of larval zebrafish reveals a prominent expression of several *mglurs*. While the riboprobes of group II and III *mglurs* 2*b*, -4, -6*b*, -7, and -8*a* only partially stain the OB, *mglur2b* and -8*b* are highly expressed in this structure. Expression of some *mglurs* in this region, for instance *mglurs* 2*b* and -8*b*, are highly similar, suggesting that many neurons of the OB express a variety of *mglur* subtypes. In addition, in the innermost layer of the OB in adult fish all four group I *mglur* genes are expressed. Therefore, the granule and periglomerular cells, located in this area, likely express different types of the group I *mglurs*. In contrast to that, larval tissue only reveals expression of the group I *mglurs* 1*a* and -5*b* in the OB, suggesting that the other two members function in the adult but not during development.

The finding of such a variety of *mglurs* in the zebrafish OB is comparable to the situation in mammals, where expression for all mGluRs has been described (Dong et al., 2009; Vardi et al., 2011). Similar to our results in adult zebrafish, group I mGluRs are highly expressed in the granule cell layer of the rodent OB (Romano et al., 1995; Shigemoto et al., 1993; Sahara et al., 2001), where they likely participate in regulating the excitability of neurons (Jian et al., 2010; Heinbockel et al., 2007). In contrast to zebrafish, group I mGluRs are additionally located in other layers of the mammalian OB bulb (Shigemoto et al., 1992; Heinbockel et al., 2004; Sahara et al., 2001), mostly on mitral cells (Nakanishi and Masu, 1994; Saugstad et al., 1997; Ohishi et al., 1993; Sahara et al., 2001; Kinzie et al., 1997; Duvoisin et al., 1995) that convey information from the OB to higher brain regions.

Optic tectum

Organized in a layered structure, the optic tectum (TeO) mediates complex visually evoked behaviors by integrating multisensory information (Nevin *et al.*, 2010). Most retinal

afferents enter the uppermost tectal layer where they make excitatory glutamatergic synaptic contact with interneurons (Kageyama and Meyer, 1989; van Keuren-Jensen and Cline, 2006). These send the information along vertically oriented dendrites into deeper tectal areas via inhibitory GABAergic or excitatory glutamatergic or cholinergic synapses (Kinoshita and Ito, 2006). As information is highly processed and filtered within tectal layers, recent research focused more on the investigation of distinct microcircuits (e.g. Del Bene *et al.*, 2010; Ramdya and Engert, 2008). However, to understand circuit formation and processing in this multilaminar structure, it is necessary to know about its general features such as neuron- or receptor subtypes.

Besides *mglur2b* and *-6b*, all *mglur* subtypes show expression in the TeO of larval zebrafish. While group I *mglurs* are still expressed in the TeO at 5 dpf, they reveal a stronger and broader expression in three-day-old larvae, suggesting tight developmental regulation. In the adult zebrafish TeO group I *mglurs* are expressed at varying intensities in the layers of the periventricular grey zone (PGZ). Noteworthy is the prominent expression of *mglur1b* in the superficial gray/fibrous layer (SFGS). Group II *mglur2a* and *-3* show a faint expression and group III *mglur4*, *-6a*, *-7* and both *mglur8* paralogs are strongly expressed in the TeO. Most published studies discuss expression but not function of mGluR family members in the superior colliculus, which is homologous to the TeO of non-anamniote vertebrates (e.g. Ohishi *et al.*, 1993; Kinoshita *et al.*, 1998; Cirone *et al.*, 2002b). At the functional level a contribution of group I, II, and III mGluRs to the modulation of synaptic signaling in the superior colliculus of rats (Cirone and Salt 2000, 2001; Cirone *et al.*, 2002a; White *et al.*, 2003) has been reported. However, except for a study demonstrating that visually evoked mGluR1-activation indirectly affects AMPAR synaptic plasticity, no functional details are known (van Keuren-Jensen and Cline, 2006).

Hypothalamus

The hypothalamus, located in the ventral part of the diencephalon, is the central organizing structure for the regulation of homeostatic functions. Various studies report the presence of mGluRs in the mammalian hypothalamus (Kiss et al., 1996; Schrader and Tasker, 1997; Chen and van den Pol, 1998; Pampillo et al., 2002; Panatier et al., 2004), implying a significant role for them in the modulation of signal transduction and thereby influencing homeostasis (reviewed in Kuzmiski and Bains, 2010). Given the conserved hypothalamic development of teleosts and mammals (Machluf et al., 2011), fish represent a suitable model to study hypothalamic function and signaling.

In larval zebrafish a variety of *mglurs* are expressed in hypothalamic regions (*mglur1a*, -*2b*, -*3*, -*5a*, -*5b*, and all group III *mglurs*). Amongst them, the members of group I mGluRs seem to play crucial roles in various areas of the hypothalamus pathways (Caruso et al., 2006; Huang and van den Pol, 2007). Both *mglur5* paralogs reveal a very prominent and defined expression in the rostral and intermediate hypothalamus as well as in the torus lateralis and the inferior lobe. In rats a physiological study demonstrates an influence of astrocytic group I mGluRs on synaptic plasticity of neighboring hypothalamic neurons (Gordon et al., 2009). Since the expression of both mGluR1 and -5 in hypothalamic glia cells is described (Silva et al., 1999; van den Pol et al., 1995), it is likely that both receptors mediate plasticity. Besides that, group I mGluRs influence signal transduction in many other hypothalamic signaling pathways (Caruso et al., 2006; Huang and van den Pol, 2007; Dewing et al., 2007). The low expression of *mglur1b* in only a few adult hypothalamic structures indicates that in zebrafish the major role in hypothalamic signal modulation is likely controlled by mGluR1a, and the two mGluR5 paralogs.

Group II *mglur2b* and -*3* are strongly expressed in the caudal hypothalamus. However, neither an involvement in the modulation of GABA release in suprachiasmatic neurons of rats

(Chen and van den Pol, 1998) no other specific functions have been described in the literature. Although expression of all group III mGluRs in the hypothalamus is described (Ghosh et al., 1997; Chen and van den Pol, 1998), specific functions for receptor subtypes are unknown due to the lack of specific agonists (Schrader and Tasker, 1997; Chen and van den Pol, 1998; Panatier et al., 2004; Kuzmiski et al., 2009). The mRNA expression analysis demonstrates that all group III *mglurs* are present in the larval zebrafish hypothalamus albeit with a variation of the regions and the expression level.

Cerebellum

The most extensively studied mGluR in the cerebellum of mammals is mGluR1. Its knockout leads to problems in motor coordination and spatial learning (Aiba et al., 1994b; Conquet et al., 1994), demonstrating a crucial role in long term potentiation and –depression (Aiba et al., 1994b; Conquet et al., 1994). Consistent with that we find prominent expression for both *mglur1* paralogs in the zebrafish cerebellum. At larval stages the cerebellar *mglur1a*-riboprobe detects cells that resemble a Purkinje cell staining of an antibody against Parvalbumin7 (Bae et al., 2009). In contrast to Parvalbumin 7, *mglur1b* is additionally expressed in a subpopulation of granule cells in the dorsal and lateral part of the cerebellum. The staining pattern for *mglur1b* seems to coincide partially with the vesicular glutamate transporter 1 (Vglut1), arguing for a presynaptic function as observed for Vglut1 (Bae et al., 2009). Expression of *mglur1s* in these regions persists into adulthood where both *mglur1* transcripts are present in Purkinje cells, and the granule cell layer of the corpus cerebelli as well as the eminentia granularis are *mglur1b*-positive. Interestingly, human mRNA expression studies of *mglur1* splice variants show only expression for certain variants in cerebellar granule, Purkinje and basket cells, whereas another isoform is exclusively present in granule cells (Makoff et al., 1997). This

suggests that the cerebellar subdivision of zebrafish *mglur1* paralogs may be similar to the subdivision of *mglur1* splice variants in humans, even though in zebrafish the two variants originate from a whole genome duplication event and not from the same gene. The expression of *mglur1* in corresponding structures in fish and mammals may indicate some conservation in functional aspects of these receptors. While the ancestral gene presumably fulfilled the combined function of both paralogs, functions may have later been subdivided between the two paralogs by subfunctionalization (Force et al., 1999). Zebrafish *mglur1* paralogs are also expressed in regions of the brain where the corresponding mammalian ortholog might not be expressed. However, as *mglur1* variants have a very broad expression range it remains to be determined whether some of the zebrafish mRNA patterns are due to newly acquired gene functions.

Similar to mGluR1, the second group I mGluR, mGluR5, is also involved in synaptic plasticity of the cerebellum, although not in exactly the same way as already apparent when comparing the different phenotypes of knockout mice (Aiba et al., 1994b; Conquet et al., 1994; Lu et al., 1997). In line with that we find *mglur5a* and *-5b* expression in the larval and adult zebrafish cerebellum, albeit not in such a broad manner as for the *mglur1* paralogs. Moreover, except for *mglur6b*, we localize all *mglur* subtypes in at least parts of the cerebellar structures of zebrafish larvae. Since both group II (Ghose et al., 1997; Knoflach and Kemp, 1998; Watanabe and Nakanishi, 2003) and group III mGluRs are known to act in cerebellar signaling pathways (Pekhlitski et al., 1996; Cartmell et al., 1997; Abitbol et al., 2008; Zhang et al., 2008), and are assumed to be involved in synaptic plasticity and motor learning (Anwyl, 1999; Zhu et al., 2005) the presence of transcripts for these *mglur* subtypes in the zebrafish cerebellum are expected. However, an exception is mGluR6, which is expressed in lateral cerebellar regions of 5-day-old zebrafish larvae but has not been reported yet to be present in developing or mature cerebellar structures in mammals (Berthele et al., 1999).

Retina

Since glutamate is the main neurotransmitter in the retina, various mGluRs can be found in the eye of vertebrates, where they have been shown to be involved in signal modulation at the first visual synapse (Rothe et al., 1994; Linn and Gafka, 1999; Higgs et al., 2002; Higgs and Lukasiewicz, 2002; Hirasawa et al., 2002; Hosoi et al., 2005; Fan and Yazulla, 2007). However, we found expression for most zebrafish *mglurs* in the inner retina but not in the outermost layers where photoreceptors (ONL) and horizontal cell bodies (outermost INL close to OPL) are located. Both *mglur1* paralogs are expressed in inner retinal layers of larval zebrafish. While we could not detect any *mglur5a* transcripts in the larval retina, *mglur5b* shows a faint expression in the inner retina at 3 but not at 5 dpf. Expression of *mglur1a* and *-5b* is consistent between embryonic and adult tissue, whereas *mglur1b* and *-5a* are additionally expressed in the medial INL in adult retinas, indicating an additional role for these genes in adulthood. An immunohistochemical study performed in adult goldfish retinas showed expression of mGluR1 α mainly in ON-bipolar cell dendrites (Joselevitch et al., 2007). This study also reported mGluR5 immunoreactivity in glia cells and in the OPL, INL, and IPL. While the expression pattern for zebrafish *mglur1b* is consistent with the one found for goldfish mGluR1 α , the expression we found for the *mglur5* paralogs is concentrated on the INL and does not resemble glia cell labeling. However, a direct comparison to our results is not possible since the antibodies used by Joselevitch and colleagues were not specifically designed against teleost tissue and therefore also not against a specific mGluR paralog.

Group II mGluRs are involved in the presynaptic inhibition of retinotectal transmission in goldfish (Zhang and Schmidt, 1999) which explains the presence of two of the three zebrafish group II mGluRs in the ganglion cell layer. However, as mGluR3 is – in contrast to our data – not

expressed in the retina of cat and goldfish (Joselevitch et al., 2007; Cai and Pourcho, 1999), and as we could not find *mglur2b*-positive labeling in the larval fish retina, this function seems to be exclusively mediated by zebrafish mGluR2a. In addition, the expression of *mglur2* mRNA in rat amacrine and ganglion cells (Hartveit et al., 1995) agrees well with the expression of *mglur2a* in the proximal INL and GCL.

Other interesting differences in expression exist for the *mglur6* paralogs: Vertebrate mGluR6 is known to be involved in the ON-bipolar cell signaling being exclusively expressed in ON-bipolar cell dendrites (Hartveit et al., 1995; Masu et al., 1995; Nomura et al., 1994; Ueda et al., 1997; Vardi et al., 2000; Vardi and Morigiwa, 1997). We only found expression of *mglur6b* in the medial INL, where bipolar cell bodies are located, whereas *mglur6a* is not expressed in this layer at larval stages. This suggests that the known function in the ON-bipolar cell pathway of the mammalian mGluR6 is fulfilled exclusively by the zebrafish mGluR6b in the larval retina. The expression of both *mglur6* paralogs in the retinal bipolar cell layer of adult zebrafish (Huang et al., 2012) suggests – at least for *mglur6a* – a developmental regulation of gene expression in this retinal cell layer. *mglur6* paralogs may have separate roles in the ON-bipolar cell pathway. Since rods mature later than cones (Branchek and Bremiller 1984), one could envision a scenario where the cone ON-pathway mainly relies on *mglur6b* and the rod ON-pathway on *mglur6a*, which is not expressed until rods are functionally mature. mGluR6 was thought of being exclusively involved in the postsynaptic reception of the ON-pathway signal, however, our finding of *mglur6* expression in the inner retina proposes an additional modulatory role (Huang et al., 2012). The fact that *mglur6* was recently detected in human retinal ganglion cell bodies (Klooster et al., 2011) indicates that this role might be more widespread in vertebrates than originally anticipated.

The photopic retina of zebrafish is already highly developed and nearly fully functional at 5 dpf, when rods just begin to functionally integrate into the retina at that stage (Branchek,

1984; Branchek and Bremiller, 1984). A developmental regulation of gene expression – as proposed for *mglur6b* and the *mglur8* paralogs – could explain the restricted expression pattern we find for most *mglurs*. Except for mGluR6 that directly acts as glutamate receptor in the ON-bipolar cell pathway (Masu et al., 1995), all other mGluRs are known to indirectly modulate synaptic signaling in the retina at pre- and postsynapses, for example by changing presynaptic calcium concentration in photoreceptor synaptic terminals (Koulen et al., 1999; Akopian and Witkovsky, 1996) or even by directly influencing transmitter release (Hirasawa et al., 2002). However, the expression of almost all zebrafish *mglurs* in the retina shows their significance in visual signal transmission.

Conclusion

Glutamate mediates most of the excitatory signaling of the nervous system and is of prime importance for the functioning of neuronal circuits. Knowledge of glutamatergic signaling, in particular of its receptors, helps deciphering underlying mechanisms and organizations of these circuits. Our detailed analysis of zebrafish metabotropic glutamate receptors showed comparably broad expression in similar regions as their mammalian orthologs, implicating their significance in diverse neuronal functions. The paralogous zebrafish *mglurs* are all expressed in overlapping but also in exclusive brain regions, suggesting functional specialization. Moreover, the high coincidence between larval and adult gene expression makes larval zebrafish suitable as a model to study mGluR function and may help to facilitate the disentanglement of neuronal circuits.

Abbreviations

A	anterior thalamic nucleus
ad	adult
ac	anterior commissure
ALLG	anterior lateral line ganglion
CC	cerebellar crest
CCe	corpus cerebelli
CeP	cerebellar plate
CePl	lateral part of cerebellar plate
CNS	central nervous system
CIL	central nucleus of inferior lobe
CM	corpus mamillare
CNS	central nervous system
CP	central posterior thalamic nucleus
Cpop	commissura postoptica
D	dorsal telencephalic area
Dc	central zone of D
Dd	dorsal zone of D
DIL	diffuse nucleus of inferior lobe
DI	lateral zone of D
Dm	medial zone of D
DON	descending octaval nucleus
Dp	posterior zone of D
DP	dorsal posterior thalamic nucleus
DT	dorsal thalamus
E	epiphysis
EG	eminentia granularis
EmT	eminentia thalami
ET	entopeduncular nucleus
FG	facial ganglion
FL	facial lobe
GCL	ganglion cell layer (retina); granule cell layer (cerebellum)
GG	glossopharyngeal ganglion
GT	griseum tectale
Ha	habenula
Hc (larvae)	caudal hypothalamus
Hc (adult)	caudal zone of periventricular hypothalamus
Hd	dorsal zone of periventricular hypothalamus
Hi	intermediate hypothalamus
Hr	rostral hypothalamus
Hv	ventral zone of periventricular hypothalamus
INL	inner nuclear layer
IO	inferior olive
l	lateral tectal proliferation zone
LCa	lobus caudalis cerebellum
lfb	lateral forebrain bundle

LH	lateral hypothalamic nucleus
LVe	lateral ventricular recess of hypothalamus
mn	motor neurons
ML	molecular layer (cerebellum)
MC	Mauthner cells
MO	medulla oblongata
MOc	caudal part of MO
MOl	lateral part of MO
MO _n	nucleus in MO
MON	medial octavolateralis nucleus
MO _p	posterior part of MO
M2	migrated posterior tubercular area
M3	migrated area of EmT
N _{ln}	nucleus interpeduncularis
NLV	nucleus lateralis valvulae
OA	octaval area
OB	olfactory bulb
OG	otic ganglion
ON	optic nerve
P	pallium
PCL	Purkinje cell layer (cerebellum)
PGa	anterior preglomerular nucleus
PGl	lateral preglomerular nucleus
PG _m	medial preglomerular nucleus
PGZ (L1/2, L3)	periventricular gray zone of TeO (layer 1/2, layer 3)
PLL _G	posterior lateral line ganglion
Po	preoptic region
poc	postoptic commissure
PPa	parvocellular preoptic nucleus, anterior part
PP _p	parvocellular preoptic nucleus, posterior part
Pr	pretectum
PSp	parvocellular superficial pretectal nucleus
PT	posterior tuberculum
PT _d	dorsal part of posterior tuberculum
PTN	posterior tubercular nucleus
PT _v	ventral part of posterior tuberculum
PVe	posterior recess ventricle of hypothalamus
RF	reticular formation
RVe	rhombencephalic ventricle
PVO	paraventricular organ
S	subpallium
S _d	dorsal division of subpallium
SFGS	superficial gray/fibrous layer of TeO
SO	optic layer of TeO
SR	superior raphe
S _v	ventral division of subpallium
T	midbrain tegmentum

TeO	tectum opticum
TeVe	tectal ventricle
TG	trigeminal ganglion
TL	torus longitudinalis
TLa	torus lateralis
TN	tegmental nucleus
TPp	periventricular nucleus of PT
TS	torus semicircularis
TVe	telencephalic ventricle
Va	valvula cerebelli
Val/m	lateral/medial division of Va
V	ventral telencephalic area
Vc	central nucleus of V
VG	vagal ganglion
VI	lateral nucleus of V
VL	vagal lobe
VM	ventromedial thalamic nucleus
VT	ventral thalamus
Vv	ventral nucleus of V

Acknowledgements

We thank Ying-Yu Huang for initial help with the study and we are grateful to Kara Dannenhauer and the other members of the fish facility team for excellent animal care. This work was supported by the Swiss Science Foundation (31003A_135598/1) and the EU 7th frame with program RETICIRC, ZF-HEALTH. In addition, Thomas Mueller was supported by the European Commission (FP7, ZF-HEALTH 242048).

Author's Contribution

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. MFH: cloning, expression analysis, drafting and revision of the manuscript. MG: gene annotation and phylogenetic analysis, revision of the manuscript. TM: expert advice in naming brain structures, revision of the manuscript. SCFN: study supervision, concept, and design, revision of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

References

- Abitbol, K, Acher, F, Daniel, H. 2008. Depression of excitatory transmission at PF-PC synapse by group III metabotropic glutamate receptors is provided exclusively by mGluR4 in the rodent cerebellar cortex. *J. Neurochem*, 105, 2069–2079.
- Aiba, A, Chen, C, Herrup, K, Rosenmund, C, Stevens, CF, Tonegawa, S. 1994a. Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell*, 79, 365–375.
- Aiba, A, Kano, M., Chen, C, Stanton, ME, Fox, GD, Herrup, K., Zwingman, TA, Tonegawa, S. 1994b. Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. *Cell*, 79, 377–388.
- Akopian, A, Witkovsky, P. 1996. Activation of metabotropic glutamate receptors decreases a high-threshold calcium current in spiking neurons of the *Xenopus* retina. *Vis. Neurosci*, 13, 549–557.
- Amsterdam, A, Lin, S, Hopkins, N. 1995. The *Aequorea victoria* green fluorescent protein can be used as a reporter in live zebrafish embryos. *Dev Biol*, 171, 123–129.
- Anisimova, M, Gascuel, O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst. Biol*, 55, 539–552.
- Anwyl, R. 1999. Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. *Brain Res. Brain Res. Rev*, 29, 83–120.
- Bae, Y-K, Kani, S, Shimizu, T, Tanabe, K, Nojima, H, Kimura, Y, Higashijima, S-I, Hibi, M. 2009. Anatomy of zebrafish cerebellum and screen for mutations affecting its development. *Dev. Biol*, 330, 406–426.
- Baude, A, Nusser, Z, Roberts, JD, Mulvihill, E, McIlhinney, RA, Somogyi, P. 1993. The metabotropic glutamate receptor (mGluR1 α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron*, 11, 771–787.
- Berthele, A, Platzer, S, Laurie, D.J., Weis, S, Sommer, B, Zieglgänsberger, W, Conrad, B, Tölle, TR. 1999. Expression of metabotropic glutamate receptor subtype mRNA (mGluR1-8) in human cerebellum. *Neuroreport*, 10, 3861–3867.
- Branchek, T. 1984. The development of photoreceptors in the zebrafish, *brachydanio rerio*. II. Function. *J. Comp. Neurol*, 224, 116–122.
- Branchek, T, Bremiller, R. 1984. The development of photoreceptors in the zebrafish, *Brachydanio rerio*. I. Structure. *J Comp Neurol*, 224, 107–115.
- Brandstätter, JH, Koulen, P, Kuhn, R., van der Putten, H, Wässle, H. 1996. Compartmental localization of a metabotropic glutamate receptor (mGluR7): two different active sites at a retinal synapse. *J Neurosci*, 16, 4749–4756.
- Byrnes, KR, Loane, DJ, Faden, AI. 2009. Metabotropic glutamate receptors as targets for multipotential treatment of neurological disorders. *Neurotherapeutics*, 6, 94–107.
- Cai, W, Pourcho, RG. 1999. Localization of metabotropic glutamate receptors mGluR1 α and mGluR2/3 in the cat retina. *J. Comp. Neurol*, 407, 427–437.
- Cartmell, J, Kemp, JA, Mutel, V. 1997. L-AP4 inhibition of depolarization-evoked cGMP formation in rat cerebellum. *Neurosci. Lett*, 228, 191–194.
- Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol*, 17, 540–552.
- Chen, G, van den Pol, AN. 1998. Coexpression of multiple metabotropic glutamate receptors in axon terminals of single suprachiasmatic nucleus neurons. *J. Neurophysiol*, 80, 1932–1938.
- Cirone, J, and Salt, TE. 2000. Physiological role of group III metabotropic glutamate receptors in visually responsive neurons of the rat superficial superior colliculus. *Eur. J. Neurosci*, 12, 847–855.
- Cirone, J, Salt, TE. 2001. Group II and III metabotropic glutamate receptors contribute to different aspects of visual response processing in the rat superior colliculus. *J. Physiol. (Lond.)*, 534, 169–178.

- Cirone, J, Potheary, CA, Turner, JP, Salt, TE. 2002a. Group I metabotropic glutamate receptors (mGluRs) modulate visual responses in the superficial superior colliculus of the rat. *J. Physiol. (Lond.)*, 541, 895–903.
- Cirone, J, Sharp, C, Jeffery, G, Salt, TE. 2002b. Distribution of metabotropic glutamate receptors in the superior colliculus of the adult rat, ferret and cat. *Neuroscience*, 109, 779–786.
- Conquet, F, Bashir, ZI, Davies, CH, Daniel, H, Ferraguti, F, Bordi, F, Franz-Bacon, K, Reggiani, A, Matarese, V, Condé, F. 1994. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature*, 372, 237–243.
- Corrêa, SAL, Zupanc, GKH. 2004. Re-evaluation of the afferent connections of the pituitary in the weakly electric fish *Apteronotus leptorhynchus*: an in vitro tract-tracing study. *J. Comp. Neurol.*, 470, 39–49.
- Del Bene, F, Wyart, C, Robles, E, Tran, A, Looger, L, Scott, EK, Isacoff, EY, Baier, H. 2010. Filtering of visual information in the tectum by an identified neural circuit. *Science*, 330, 669–673.
- Dereeper, A, Guignon, V, Blanc, G, Audic, S, Buffet, S, Chevenet, F, Dufayard, JF, Guindon, S, Lefort, V, Lescot, M, Claverie, JM, Gascuel, O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res*, 36, W465–9.
- Dewing, P, Boulware, MI, Sinchak, K, Christensen, A, Mermelstein, PG, Micevych, P. 2007. Membrane estrogen receptor- α interactions with metabotropic glutamate receptor 1 α modulate female sexual receptivity in rats. *J. Neurosci*, 27, 9294–9300.
- Dong, H-W, Heinbockel, T, Hamilton, KA, Hayar, A, Ennis, M. 2009. Metabotropic glutamate receptors and dendrodendritic synapses in the main olfactory bulb. *Ann. N. Y. Acad. Sci*, 1170, 224–238.
- Duvoisin, RM, Zhang, C, Ramonell, K. 1995. A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb. *J Neurosci*, 15, 3075–3083.
- Edgar, RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 113.
- Fan, S-F, Yazulla, S. 2007. Retrograde endocannabinoid inhibition of goldfish retinal cones is mediated by 2-arachidonoyl glycerol. *Vis. Neurosci.*, 24, 257–267.
- Ferraguti, F, Conquet, F, Corti, C, Grandes, P, Kuhn, R, Knopfel, T. 1998. Immunohistochemical localization of the mGluR1 β metabotropic glutamate receptor in the adult rodent forebrain: evidence for a differential distribution of mGluR1 splice variants. *J. Comp. Neurol.*, 400, 391–407.
- Ferraguti, F, Cobden, P, Pollard, M, Cope, D, Shigemoto, R, Watanabe, M, Somogyi, P. 2004. Immunolocalization of metabotropic glutamate receptor 1 α (mGluR1 α) in distinct classes of interneuron in the CA1 region of the rat hippocampus. *Hippocampus*, 14, 193–215.
- Ferraguti, F. and Shigemoto, R. 2006. Metabotropic glutamate receptors. *Cell Tissue Res.*, 326, 483–504.
- Force, A, Lynch, M, Pickett, FB, Amores, A, Yan, YL, Postlethwait, J. 1999. Preservation of duplicate genes by complementary, degenerative mutations. *Genetics*, 151, 1531–1545.
- Gesemann, M, Lesslauer, A, Maurer, CM, Schönthaler, HB, Neuhaus, SCF. 2010. Phylogenetic analysis of the vertebrate excitatory/neutral amino acid transporter (SLC1/EAAT) family reveals lineage specific subfamilies. *BMC Evol. Biol*, 10, 117.
- Ghose, S, Wroblewska, B, Corsi, L, Grayson, DR, de Blas, AL, Vicini, S, Neale, JH. 1997. N-acetylaspartylglutamate stimulates metabotropic glutamate receptor 3 to regulate expression of the GABA(A) α 6 subunit in cerebellar granule cells. *J. Neurochem*, 69, 2326–2335.
- Gil-Sanz, C, Delgado-García, JM, Fairén, A and Gruart, A. 2008. Involvement of the mGluR1 receptor in hippocampal synaptic plasticity and associative learning in behaving mice. *Cereb. Cortex*, 18, 1653–1663.
- Gordon, GRJ, Iremonger, KJ, Kantevari, S, Ellis-Davies, GCR, MacVicar, BA, Bains, JS. 2009. Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. *Neuron*, 64, 391–403.
- Guindon, S, Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol*, 52, 696–704.
- Hartveit, E, Brandstätter, JH, Enz, R, Wässle, H. 1995. Expression of the mRNA of seven metabotropic glutamate receptors (mGluR1 to 7) in the rat retina. An in situ hybridization study on tissue sections and isolated cells. *Eur J Neurosci*, 7, 1472–1483.

- Heinbockel, T, Laaris, N, Ennis, M. 2007. Metabotropic glutamate receptors in the main olfactory bulb drive granule cell-mediated inhibition. *J. Neurophysiol*, 97, 858–870.
- Heinbockel, T, Heyward, P, Conquet, F, Ennis, M. 2004. Regulation of main olfactory bulb mitral cell excitability by metabotropic glutamate receptor mGluR1. *J. Neurophysiol.*, 92, 3085–3096.
- Higgs, MH, Lukasiewicz, PD. 2002. Activation of group II metabotropic glutamate receptors inhibits glutamate release from salamander retinal photoreceptors. *Vis. Neurosci*, 19, 275–281.
- Higgs, MH, Romano, C, Lukasiewicz, PD. 2002. Presynaptic effects of group III metabotropic glutamate receptors on excitatory synaptic transmission in the retina. *Neuroscience*, 115, 163–172.
- Hirasawa, H, Shiells, R, Yamada, M. 2002. A metabotropic glutamate receptor regulates transmitter release from cone presynaptic terminals in carp retinal slices. *J. Gen. Physiol*, 119, 55–68.
- Hosoi, N, Arai, I, Tachibana, M. 2005. Group III metabotropic glutamate receptors and exocytosed protons inhibit L-type calcium currents in cones but not in rods. *J. Neurosci*, 25, 4062–4072.
- Huang, H, Luo, DG, Shen, Y, Zhang, AJ, Yang, R, Yang, XL. 2004. AMPA receptor is involved in transmission of cone signal to ON bipolar cells in carp retina. *Brain Res*, 1002, 86–93.
- Huang, H, van den Pol, AN. 2007. Rapid direct excitation and long-lasting enhancement of NMDA response by group I metabotropic glutamate receptor activation of hypothalamic melanin-concentrating hormone neurons. *J. Neurosci*, 27, 11560–11572.
- Huang, Y-Y, Haug, MF, Gesemann, M, Neuhauss, SCF. 2012. Novel Expression Patterns of Metabotropic Glutamate Receptor 6 in the Zebrafish Nervous System. *PLoS ONE*, 7, 135–143.
- Jian, K, Cifelli, P, Pignatelli, A, Frigato, E, Belluzzi, O. 2010. Metabotropic glutamate receptors 1 and 5 differentially regulate bulbar dopaminergic cell function. *Brain Res*, 1354, 47–63.
- Joselevitch, C, Klooster, J, Kamermans, M. 2007. Localization of metabotropic glutamate receptors in the outer plexiform layer of the goldfish retina. *Cell Tissue Res*, 330, 389–403.
- Kageyama, GH, Meyer, RL. 1989. Glutamate-immunoreactivity in the retina and optic tectum of goldfish. *Brain Res.*, 503, 118–127.
- Kawakami, K, Shima, A. 1999. Identification of the Tol2 transposase of the medaka fish *Oryzias latipes* that catalyzes excision of a nonautonomous Tol2 element in zebrafish *Danio rerio*. *Gene*, 240, 239–244.
- Kikuta, H, Laplante, M, Navratilova, P, Komisarczuk, AZ, Engstrom, PG, Fredman, D, Akalin, A, Caccamo, M, Sealy, I, Howe, K, Ghislain, J, Pezeron, G, Mourrain, P, Ellingsen, S, Oates, AC, Thisse, C, Thisse, B, Foucher, I, Adolf, B, Geling, A, Lenhard, B, Becker, TS. 2007. Genomic regulatory blocks encompass multiple neighboring genes and maintain conserved synteny in vertebrates. *Genome Research*, 17, 545–555.
- Kinoshita, A, Shigemoto, R, Ohishi, H, van der Putten, H, Mizuno, N. 1998. Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: a light and electron microscopic study. *J. Comp. Neurol*, 393, 332–352.
- Kinoshita, M., Ito, E. 2006 Roles of periventricular neurons in retinotectal transmission in the optic tectum. *Prog. Neurobiol.*, 79, 112–121.
- Kinzie, JM, Shinohara, MM, van den Pol, AN, Westbrook, GL, Segerson, TP. 1997. Immunolocalization of metabotropic glutamate receptor 7 in the rat olfactory bulb. *J. Comp. Neurol*, 385, 372–384.
- Kiss, J, Görcs, TJ, Kuhn, R, Knöpfel, T, Csáky, A, Halász, B. 1996. Distribution of metabotropic glutamate receptor 1a in the rat hypothalamus: an immunocytochemical study using monoclonal and polyclonal antibody. *Acta. Biol. Hung*, 47, 221–237.
- Klooster, J, Yazulla, S, Kamermans, M. 2009. Ultrastructural analysis of the glutamatergic system in the outer plexiform layer of zebrafish retina. *J Chem Neuroanat*, 37, 254–265.
- Klooster, J, Blokker, J, Ten Brink, JB, Unmehopa, U, Fluiter, K, Bergen, AAB, Kamermans, M. 2011. Ultrastructural Localization and Expression of Trpm1 in the Human Retina. *Invest. Ophthalmol. Vis. Sci.*
- Knackstedt, LA, Kalivas, PW. 2009. Glutamate and reinstatement. *Curr Opin Pharmacol*, 9, 59–64.

- Knoflach, F, Kemp, JA. 1998. Metabotropic glutamate group II receptors activate a G protein-coupled inwardly rectifying K⁺ current in neurones of the rat cerebellum. *J. Physiol. (Lond.)*, 509 (Pt 2), 347–354.
- Koulen, P, Malitschek, B, Kuhn, R, Wässle, H, Brandstätter, JH. 1996. Group II and group III metabotropic glutamate receptors in the rat retina: distributions and developmental expression patterns. *Eur J Neurosci*, 8, 2177–2187.
- Koulen, P, Kuhn, R, Wässle, H, Brandstätter, JH. 1997. Group I metabotropic glutamate receptors mGluR1alpha and mGluR5a: localization in both synaptic layers of the rat retina. *J Neurosci*, 17, 2200–2211.
- Koulen, P, Kuhn, R, Wässle, H, Brandstätter, JH. 1999. Modulation of the intracellular calcium concentration in photoreceptor terminals by a presynaptic metabotropic glutamate receptor. *Proc. Natl. Acad. Sci. U.S.A.*, 96, 9909–9914.
- Kuzmiski, JB, Bains, JS. 2010. Metabotropic glutamate receptors: gatekeepers of homeostasis. *J. Neuroendocrinol*, 22, 785–792.
- Kuzmiski, JB, Pittman, QJ, Bains, JS. 2009. Metaplasticity of hypothalamic synapses following in vivo challenge. *Neuron*, 62, 839–849.
- Li, W-H, Yang, J, Gu, X. 2005. Expression divergence between duplicate genes. *Trends Genet*, 21, 602–607.
- Lillesaar, C. 2011 The serotonergic system in fish. *J. Chem. Neuroanat.*, 41, 294–308.
- Linn, CL, Gafka, AC. 1999. Activation of metabotropic glutamate receptors modulates the voltage-gated sustained calcium current in a teleost horizontal cell. *J. Neurophysiol.*, 81, 425–434.
- Lu, YM, Jia, Z, Janus, C, Henderson, JT, Gerlai, R, Wojtowicz, JM, Roder, JC. 1997. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J. Neurosci.*, 17, 5196–5205.
- Luscher, C, Huber, KM. 2010. Group I mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. *Neuron*, 65, 445–459.
- Machluf, Y, Gutnick, A, Levkowitz, G. 2011. Development of the zebrafish hypothalamus. *Ann. N. Y. Acad. Sci.*, 1220, 93–105.
- Makoff, AJ, Phillips, T, Pilling, C, Emson, P. 1997. Expression of a novel splice variant of human mGluR1 in the cerebellum. *Neuroreport*, 8, 2943–2947.
- Martin, LJ, Blackstone, CD, Haganir, RL, Price, DL. 1992. Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron*, 9, 259–270.
- Masu, M, Iwakabe, H, Tagawa, Y, Miyoshi, T, Yamashita, M, Fukuda, Y, Sasaki, H, Hiroi, K, Nakamura, Y, Shigemoto, R, et al. 1995. Specific deficit of the ON response in visual transmission by targeted disruption of the mGluR6 gene. *Cell*, 80, 757–765.
- Meyer, A, van de Peer, Y. 2005. From 2R to 3R: evidence for a fish-specific genome duplication (FSGD). *Bioessays*, 27, 937–945.
- Mueller, T, Wullmann, MF. 2005. *Atlas of Early Zebrafish Brain Development. A Tool for Molecular Neurogenetics*, Elsevier.
- Mueller, T, Vernier, P, Wullmann, MF. 2006. A phylotypic stage in vertebrate brain development: GABA cell patterns in zebrafish compared with mouse. *J. Comp. Neurol*, 494, 620–634.
- Mullins, MC, Hammerschmidt, M, Haffter, P, Nüsslein-Volhard, C. 1994. Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Curr Biol*, 4, 189–202.
- Nakajima, Y, Iwakabe, H, Akazawa, C, Nawa, H, Shigemoto, R, Mizuno, N, Nakanishi, S. 1993. Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J Biol Chem*, 268, 11868–11873.
- Nakanishi, S, Masu, M. 1994. Molecular diversity and functions of glutamate receptors. *Annu Rev Biophys Biomol Struct*, 23, 319–348.
- Nevin, LM, Robles, E, Baier, H, Scott, EK. 2010. Focusing on optic tectum circuitry through the lens of genetics. *BMC Biol.*, 8, 126.

- Niswender, CM, Conn, PJ. 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.*, 50, 295–322.
- Nomura, A, Shigemoto, R, Nakamura, Y, Okamoto, N, Mizuno, N, Nakanishi, S. 1994. Developmentally regulated postsynaptic localization of a metabotropic glutamate receptor in rat rod bipolar cells. *Cell*, 77, 361–369.
- Northcutt, RG. 2006. Connections of the lateral and medial divisions of the goldfish telencephalic pallium. *J. Comp. Neurol.*, 494, 903–943.
- Ohishi, H, Shigemoto, R, Nakanishi, S, Mizuno, N. 1993. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study. *J. Comp. Neurol*, 335, 252–266.
- Ohno, S. 1999. Gene duplication and the uniqueness of vertebrate genomes circa 1970–1999. *Semin Cell Dev Biol*, 10, 517–522.
- Page, RD. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci*, 12, 357–358.
- Pampillo, M, Scimonelli, T, Duvilanski, BH, Celis, ME, Seilicovich, A, Lasaga, M. 2002. The activation of metabotropic glutamate receptors differentially affects GABA and alpha-melanocyte stimulating hormone release from the hypothalamus and the posterior pituitary of male rats. *Neurosci. Lett*, 327, 95–98.
- Panatier, A, Poulain, DA, Oliet, SHR. 2004. Regulation of transmitter release by high-affinity group III mGluRs in the supraoptic nucleus of the rat hypothalamus. *Neuropharmacology*, 47, 333–341.
- Pekhletski, R, Gerlai, R, Overstreet, LS, Huang, XP, Agopyan, N, Slater, NT, Abramow-Newerly, W, Roder, JC, Hampson, DR. 1996. Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. *J. Neurosci*, 16, 6364–6373.
- Pin, JP, Duvoisin, R. 1995. The metabotropic glutamate receptors: structure and functions. *Neuropharmacology*, 34, 1–26.
- Postlethwait, JH. 2007. The zebrafish genome in context: ohnologs gone missing. *J Exp Zool B Mol Dev Evol*, 308, 563–577.
- Quignon, P, Giraud, M, Rimbault, M, Lavigne, P, Tacher, S, Morin, E, Retout, E, Valin, A-S, Lindblad-Toh, K, Nicolas, J, Galibert, F. 2005. The dog and rat olfactory receptor repertoires. *Genome Biol.*, 6, R83.
- Quraishi, S, Gayet, J, Morgans, CW, Duvoisin, RM. 2007. Distribution of group-III metabotropic glutamate receptors in the retina. *J Comp Neurol*, 501, 931–943.
- Ramdya, P, Engert, F. 2008. Emergence of binocular functional properties in a monocular neural circuit. *Nat. Neurosci.*, 11, 1083–1090.
- Ramdya, P, Engert, F. 2008. Emergence of binocular functional properties in a monocular neural circuit. *Nat. Neurosci.*, 11, 1083–1090.
- Romano, C, Sesma, MA, McDonald, CT, O'Malley, K, van den Pol, AN, Olney, JW. 1995. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J. Comp. Neurol.*, 355, 455–469.
- Rosemond, E, Wang, M, Yao, Y, Storjohann, L, Stormann, T, Johnson, EC, Hampson, DR. 2004. Molecular basis for the differential agonist affinities of group III metabotropic glutamate receptors. *Mol Pharmacol*, 66, 834–842.
- Rothe, T, Bigl, V, Grantyn, R. 1994. Potentiating and depressant effects of metabotropic glutamate receptor agonists on high-voltage-activated calcium currents in cultured retinal ganglion neurons from postnatal mice. *Pflugers Arch.*, 426, 161–170.
- Sahara, Y, Kubota, T, Ichikawa, M. 2001. Cellular localization of metabotropic glutamate receptors mGluR1, 2/3, 5 and 7 in the main and accessory olfactory bulb of the rat. *Neurosci. Lett*, 312, 59–62.
- Saugstad, JA, Kinzie, JM, Shinohara, MM, Segerson, TP, Westbrook, GL. 1997. Cloning and expression of rat metabotropic glutamate receptor 8 reveals a distinct pharmacological profile. *Mol. Pharmacol*, 51, 119–125.

- Schrader, LA, Tasker, JG. 1997. Presynaptic modulation by metabotropic glutamate receptors of excitatory and inhibitory synaptic inputs to hypothalamic magnocellular neurons. *J. Neurophysiol*, 77, 527–536.
- Schweitzer, J, Driever, W. 2009. Development of the dopamine systems in zebrafish. *Adv. Exp. Med. Biol.*, 651, 1–14.
- Sen, M, Gleason, E. 2006. Immunolocalization of metabotropic glutamate receptors 1 and 5 in the synaptic layers of the chicken retina. *Vis Neurosci*, 23, 221–231.
- Shigemoto, R, Nakanishi, S, Mizuno, N. 1992. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat. *J. Comp. Neurol.*, 322, 121–135.
- Shigemoto, R, Nomura, S, Ohishi, H, Sugihara, H, Nakanishi, S, Mizuno, N. 1993. Immunohistochemical localization of a metabotropic glutamate receptor, mGluR5, in the rat brain. *Neurosci. Lett.*, 163, 53–57.
- Silva, GA, Theriault, E, Mills, LR, Pennefather, PS, Feeney, CJ. 1999. Group I and II metabotropic glutamate receptor expression in cultured rat spinal cord astrocytes. *Neurosci. Lett*, 263, 117–120.
- Tehrani, A, Wheeler-Schilling, TH, Guenther, E. 2000. Coexpression patterns of mGluR mRNAs in rat retinal ganglion cells: a single-cell RT-PCR study. *Invest Ophthalmol Vis Sci*, 41, 314–319.
- Thisse, C, Thisse, B. 2008. High-resolution in situ hybridization to whole-mount zebrafish embryos. *Nat Protoc*, 3, 59–69.
- Ueda, Y, Iwakabe, H, Masu, M, Suzuki, M, Nakanishi, S. 1997. The mGluR6 5' upstream transgene sequence directs a cell-specific and developmentally regulated expression in retinal rod and ON-type cone bipolar cells. *J Neurosci*, 17, 3014–3023.
- van den Pol, AN, Romano, C, Ghosh, P. 1995. Metabotropic glutamate receptor mGluR5 subcellular distribution and developmental expression in hypothalamus. *J. Comp. Neurol*, 362, 134–150.
- van Keuren-Jensen, K, Cline, HT. 2006. Visual experience regulates metabotropic glutamate receptor-mediated plasticity of AMPA receptor synaptic transmission by homer1a induction. *J. Neurosci*, 26, 7575–7580.
- Vardi, N, Duvoisin, R, Wu, G, Sterling, P. 2000. Localization of mGluR6 to dendrites of ON bipolar cells in primate retina. *J Comp Neurol*, 423, 402–412.
- Vardi, N, Morigiwa, K. 1997. ON cone bipolar cells in rat express the metabotropic receptor mGluR6. *Vis Neurosci*, 14, 789–794.
- Vardi, T, Fina, M, Zhang, L, Dhingra, A, Vardi, N. 2011. mGluR6 transcripts in non-neuronal tissues. *J. Histochem. Cytochem.*, 59, 1076–1086.
- Watanabe, D, Nakanishi, S. 2003. mGluR2 postsynaptically senses granule cell inputs at Golgi cell synapses. *Neuron*, 39, 821–829.
- Wersinger, E, Schwab, Y, Sahel, JA, Rendon, A, Pow, DV, Picaud, S, Roux, MJ. 2006. The glutamate transporter EAAT5 works as a presynaptic receptor in mouse rod bipolar cells. *J Physiol*, 577, 221–234.
- Westerfield, M. 2000. The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*). 4th ed., Univ. of Oregon Press, Eugene.
- White, A-M, Kylänpää, RA, Christie, LA, McIntosh, SJ, Irving, AJ, Platt, B. 2003. Presynaptic group I metabotropic glutamate receptors modulate synaptic transmission in the rat superior colliculus via 4-AP sensitive K(+) channels. *Br. J. Pharmacol.*, 140, 1421–1433.
- Wullmann, MF, Mueller, T. 2004a. Identification and morphogenesis of the eminentia thalami in the zebrafish. *J. Comp. Neurol*, 471, 37–48.
- Wullmann, MF, Mueller, T. 2004b. Teleostean and mammalian forebrains contrasted: Evidence from genes to behavior. *J. Comp. Neurol.*, 475, 143–162.
- Wullmann, MF, Rupp, B, Reichert, H. 1996. Neuroanatomy of the Zebrafish Brain: A Topological Atlas, Birkhäuser. Yamamoto, K, Ruuskanen, JO, Wullmann, MF, Vernier, P. 2011. Differential expression of dopaminergic cell markers in the adult zebrafish forebrain. *J. Comp. Neurol*, 519, 576–598.

- Zhang, C, Schmidt, JT. (1999) Adenosine A1 and class II metabotropic glutamate receptors mediate shared presynaptic inhibition of retinotectal transmission. *J. Neurophysiol.*, 82, 2947–2955.
- Zhang, C-S, Bertaso, F, Eulenburg, V, Lerner-Natoli, M, Herin, GA, Bauer, L, Bockaert, J, Fagni, L, Betz, H, and Scheschonka, A. 2008. Knock-in mice lacking the PDZ-ligand motif of mGluR7a show impaired PKC-dependent autoinhibition of glutamate release, spatial working memory deficits, and increased susceptibility to pentylentetrazol. *J. Neurosci*, 28, 8604–8614.
- Zhu, L, Strata, P, and Andjus, PR. 2005. Pharmacology of the metabotropic glutamate receptor mediated current at the climbing fiber to Purkinje cell synapse. *Prog. Brain Res*, 148, 299–306.

For Peer Review

Figure and Table Legends

Figures

Figure 1 - Zebrafish mGluR subfamilies have retained duplicated genes. *mGluR* sequences of the following species were used in phylogenetic reconstructions (hs = *Homo sapiens*; mm = *Mus musculus* and dr = *Danio rerio*). While zebrafish *mGluRs* are shown in dark gray, mouse *mGluRs* are given in middle gray and human mGluRs in light gray. As an outgroup the *Drosophila melanogaster* (dm) mXr receptor gene, which shares about 40% homology with vertebrate *mGluRs*, was used. Note that except for mGluR3, -4 and -7 the zebrafish has retained gene duplicates for all other mGluRs. The scale bar of 0.3 corresponds to 30 % nucleotide exchange.

Figure 2 - Expression pattern of *mglur1a* in 3 and 5 day old zebrafish

Expression of *mglur1a* transcripts detected by *in situ* hybridization in 3 day old whole mounts (A-F), 5 day old whole mounts (G-J) and the respective cross sections (K-R) of the zebrafish head. At 3 dpf both the dorsal (A-C) and the lateral (D-F) views reveal strongest *mglur1a* expression in the telencephalon (OB and Sd) and the cerebellar region (Va, CeP) as well as in the retina (INL, GCL). Cross sections of 5 day old fish stained with the *mglur1a* riboprobe only reveal staining in the Sd but not in the OB (K). In addition strong expression is also found in the cerebellum (Va, CeP) in both dorsal (G) and lateral (I) views as well as in cross sections (O-Q). Levels of the cross sections K-Q are indicated in J. For abbreviations see list. Scale bar in A (applies to A-F) and in G (applies to G-J) = 50 μm , scale bars in K-R = 20 μm .

Figure 3 - Expression pattern of *mglur1b* in 3 and 5 day old zebrafish

Expression of the *mglur1b* riboprobe detected by *in situ* hybridization in 3 day old (A-E) and 5 day old (F-I) fish larvae on whole mounts as well as on cross sections of 5 day old fish heads (J-Q).

Whole mount expression of *mglur1b* in 3 day old fish in dorsal (A-C) and lateral views (D,E) shows strongest staining in the retinal ganglion cell layer (GCL) and the eminentia granularis (EG) of the cerebellum. In 5 day old fish the dorsal (F,G) and lateral (H,I) views show a similar pattern with highest expression in the eminentia granularis (EG). This expression is confirmed in cross sections (J-Q). In addition, distinctive expression of *mglur1b* is found in the lateral tectal proliferation zone (l), the epiphysis (E), and the cerebellar plate (CeP). Levels of the cross sections J-P are indicated in I. For abbreviations see list. Scale bar in A (applies to A-E) and in F (applies to F-I) = 50 μ m, scale bars in J-Q = 20 μ m.

Figure 4 - Expression pattern of group I *mglur1* paralogs in adult zebrafish brain

mglur1 expression in sagittal (A,L), retinal (B,M), and cross sections (D-K,O-T) of 5.5 month old zebrafish.

While a sagittal section (A) shows *mglur1a* labeling in the olfactory bulb (OB), the dorsal and ventral telencephalic area (D, V), the central posterior thalamic nucleus (CP), the cerebellar region (CCe with PCL, ML, and GCL, LCa) as well as the hypothalamus (Hd, Hv, Hc, DIL), cross sections (D-K) demonstrate that also lateral parts of the hypothalamic region (LH, TL; F-I) are stained. In the retina cells of the proximal INL and the GCL are stained by the *mglur1a* riboprobe (B). Prominent *mglur1b* expression in the dorsal midbrain (TeO, TL) and medial diencephalon (TPp) was seen a sagittal section (L). Compared to *mglur1a* an even broader part of the cerebellum shows *mglur1b* expression (CCe with PCL, ML, and GCL, LCa). While the INL of the retina, most likely the bipolar cells, reveals a strong labeling of *mglur1b*, expression in the retinal ganglion cell layer (GCL) is only weak (M). The cross sections (O-T) confirm the strong *mglur1b* labeling in the cerebellum (CCe with PCL, ML and GCL, LCa, EG; R-T). They additionally show *mglur1b* expression in the caudal (Hc; R) and dorsal (Hd; S) hypothalamus.

The levels of the sagittal sections are illustrated at the bottom right of A and L. Note that the level of section A and L is slightly parasagittal. Levels of the cross sections are indicated in C and N.

All scale bars = 100 μ m.

Figure 5 - Expression pattern of *mglur5a* in 3 and 5 day old zebrafish

Dorsal (A-D,G,H) and lateral (E,F,I,J) views of *mglur5a* expression in 3 (A-F) and 5 (G-J) day old whole mounts and in cross sections of larval zebrafish at 5 dpf (K-R).

The *mglur5a* riboprobe strongly labels hypothalamic (Hr, Hi, TLa, DIL) and cerebellar structures (CeP, CePl, EG) as well as the pallium (P), the optic tectum (TeO) and the inferior olive (IO) in 3 day old fish larvae (A-F). This expression is consistent until 5 dpf where similar structures are labeled (G-J). Cross sections confirm the location of *mglur5a* in the pallium (P), the cerebellar plate (CeP), the hypothalamus (Hi, Hr, TLa, DIL), and the inferior olive (IO; K-Q).

Levels of the cross sections K-Q are indicated in J. For abbreviations see list. Scale bar in A (applies to A-F) and in G (applies to G-J) = 50 μm , scale bars in K-R = 20 μm .

Figure 6 - Expression pattern of *mglur5b* in 3 and 5 day old zebrafish

Expression of *mglur5a* transcripts detected by *in situ* hybridization in 3 day old whole mounts (A-E), 5 day old whole mounts (G-I) and the respective cross sections (J-Q) of the zebrafish head.

In dorsal (A-C) and lateral (D,E) views of 3 day old larvae stained with the *mglur5b* riboprobes a high expression in the region of the pallium (P), in hypothalamic structures (Hi, Hr, TLa, DIL), and throughout the mid- and hindbrain is seen. A weak expression is found in the inner nuclear layer of the retina (INL). At 5 dpf a similar broad distribution of *mglur5b* transcripts is visible in both dorsal (F,G) and lateral (H,I) views. Cross sections confirm the labeling of the pallium (P) and the hypothalamus (Hi, Hr, TLa, DIL) but show additional clear labeling in the olfactory bulb (OB) and in anterior parts of the diencephalon (Pr, DT, EmT, M2). Levels of the cross sections J-P are indicated in I. For abbreviations see list. Scale bar in A (applies to A-E) and in F (applies to F-I) = 50 μm , scale bars in J-Q = 20 μm .

Figure 7 - Expression pattern of group I *mglur5* paralogs in adult zebrafish brain

mglur5 expression in sagittal (A,L), retinal (B,M), and cross sections (D-K,O-W) of 5.5 month old zebrafish.

The *mglur5a* riboprobe strongly labels the telencephalic region (OB, several parts of D and V A,D-F), and layers of the optic tectum (SFGS and PGZ in TeO; A,G-K). While expression in some ventral diencephalic regions (PTN, Hd, Hv, Hc, DIL, CM) is clearly seen in a sagittal section (A), the cross sections reveal additional staining in lateral hypothalamic regions (LH, TLa, CIL; H-K). Moreover, some cell bodies of the inferior olive are *mglur5a*-positive (IO; A). A retinal cross section reveals *mglur5a* expression in the medial INL where bipolar cell bodies are expressed (B). *mglur5b* shows the most prominent expression in several areas of the dorsal telencephalon (Dc, Dd, Dl, Dm, Dp; L,O-R) and the ventral telencephalic area (Vc, Vd, Vl, Vv; B,O-Q). Similar to its paralog, *mglur5b* reveals an expression in the medial INL of the retina (M). Cross sections depict strong labeling in the olfactory bulb (OB; O). Compared to sagittal sections, a stronger labeling in hypothalamic regions is visible (Hv, Hd, Hc. LH, TLa, DIL; T-W). Moreover, the periventricular grey zone of the optic tectum (PGZ; T-W) reveals prominent *mglur5b* expression.

The levels of the sagittal sections are illustrated at the bottom right of A and L. Note that the level of section A and L is slightly parasagittal. The levels of the cross sections are indicated in C and N. All scale bars = 100 μ m.

Figure 8 - Expression pattern of *mglur2a* in 3 and 5 day old zebrafish

Expression of *mglur2a* transcripts in 3 (A-F) and 5 (G-J) day old zebrafish whole mounts depicted in dorsal (A-C,G,H) and lateral views (D-F,I,J) as well as in cross sections through the head of 5 day old whole mounts (K-R).

Expression of *mglur2a* in 3 day old fish shows prominent labeling in the olfactory bulb (OB) but also in various parts of the diencephalon and the hindbrain (A-F). At 5 dpf *mglur2a* transcripts are expressed in similar regions as in 3 day old fish (G-J). Sections of *mglur2a* hybridized 5 day old larvae (K-R) show highest expression in nuclei of the olfactory bulb nuclei (OB), in the migrated area of the eminentia thalami (M3), and in lateral cerebellar parts (CeP, EG). Besides that *mglur2a* is expressed in inner retinal layers (INL and GCL) as depicted in R. Levels of the cross sections K-Q are indicated in J. For abbreviations see list. Scale bar in A (applies to A-F) and in G (applies to G-J) = 50 μm , scale bars in K-R = 20 μm .

Figure 9 - Expression pattern of *mglur2b* in 3 and 5 day old zebrafish

In situ hybridization of 3 (A-G) and 5 (H-K) day old zebrafish larvae using the *mglur2b* riboprobe. L-S depict cross sections through a zebrafish at 5 dpf stained with *mglur2b*.

Dorsal (A-D) and lateral (E-G) views of 3 day old whole mounts show high *mglur2b* expression in the telencephalon (OB, E, P), in specific diencephalic regions (DT, Pr, T, Hi, Hc), as well as the hindbrain (CeP, MO, MC, RF). In addition expression around ventricular zones is visible (TVe, TeVe, RVe). 5 day old zebrafish tissues labeled with *mglur2b* transcripts (H-K) indicate a very similar expression pattern as at 3 dpf. The most prominent expression is found in regions around the telencephalic ventricle (TVe), in the hypothalamus (Hi, Hc), and the hindbrain (e.g. CeP, TS, RF). Cross sections of 5 day old fish stained with the *mglur2b* probe (L-S) confirm its very distinct expression profile. Levels of the cross sections L-R are indicated in K. For abbreviations see list. Scale bar in A (applies to A-G) and scale bar in H (applies to H-K) = 50 μm , scale bars in L-S = 20 μm .

Figure 10 - Expression pattern of *mglur3* in 3 and 5 day old zebrafish

Expression of *mglur3* transcripts in whole mounts at 3 (A-G) and 5 (H-K) dpf as well as in cross sections of a 5 day old larvae (L-S).

Dorsal (A-D) and lateral (E-G) views of a 3 day old zebrafish show strongest *mglur3* labeling in the pallium (P), periventricular zones (TVe, TeVe, RVe) and in hypothalamic areas (Hi, Hc). At 5 dpf expression of *mglur3* in dorsal (H,K) and lateral (J,K) views indicate a distinctive labeling in periventricular zones (TeV, TeVe, LVe) similar to the staining at 3 dpf. In addition the cerebellum (Va, CeP) and the hypothalamus are *mglur3* positive as well. Sections of an *mglur3* hybridized larvae at 5 dpf confirm the pronounced staining of all periventricular (TVe, TeVe, LVe, RVe) and some cerebellar (Va, CeP) and hypothalamic (Hi, Hc) regions. Additional expression is detected in the inferior olive (IO) and the inner retina (INL, GCL).

Levels of the cross sections L-R are indicated in K. For abbreviations see list. Scale bar in A (applies to A-G), and scale bar in H (applies to H-K) = 50 μ m, scale bars in L-S = 20 μ m.

Figure 11 - Expression pattern of *mglur4* in 3 and 5 day old zebrafish

Expression of the *mglur4* riboprobe detected by *in situ* hybridization in 3 day old (A-F) and 5 day old (G-J) fish larvae on whole mounts as well as on cross sections of 5 day old fish heads (K-R). Dorsal (A-C) and lateral (D-F) views of *mglur4* expression in 3 day old fish reveals strong staining in the cranial ganglia (TG, ALLG, FG, VG, PLLG), in the olfactory bulb (OB), and in broad regions of the diencephalon (T, TeO) and the hindbrain (EG, MO, mn). Additional expression is seen in the inner nuclear layer (INL) and the ganglion cell layer (GCL) of the retina. At 5 dpf dorsal (G,H) and lateral (I,J) views show additional expression in the habenula (Ha). The cross sections through 5 day old fish heads stained with *mglur4* probes (K-R) confirm the broad labeling of brain structures but additionally show expression in the subpallium (Sd), the preoptic region (Po), the posterior tuberculum (PT), the cerebellum (CeP) and the hypothalamus (Hr, Hi, TL_a). Levels of the cross sections K-Q are indicated in J. For abbreviations see list. Scale bar in A (applies to A-F) and scale bar in G (applies to F-J) = 50 μ m, scale bars in K-R = 20 μ m.

Figure 12 - Expression pattern of *mglur6a* in 3 and 5 day old zebrafish

Expression of *mglur6a* transcripts detected by *in situ* hybridization on whole mount larvae at 3 (A-E) and at 5 dpf (F-I) as well as in cross sections (J-Q) through the head of a 5 day old fish expressing *mglur6a*.

Whole mount expression of *mglur6a* in zebrafish larvae at 3 dpf in dorsal (A-C) and lateral views (D,E) revealing an intense labeling of the retinal ganglion cell layer (GCL) and the habenula (Ha). Dorsal (F,G) and lateral (H,I) views and cross sections (J-Q) of 5 day old fish expressing *mglur6a* show additional staining in the subpallial region (Sd), the thalamus (TeO, DT, VT, PT, T), the cerebellum (CeP), the nucleus interpeduncularis (Nln), and in two nuclei of the medulla oblongata (MO). Levels of the cross sections J-P are indicated in I. For abbreviations see list. Scale bar in A (applies to A-E) and scale bar in F (applies to F-I) = 50 μm , scale bars in J-Q = 20 μm .

Figure 13 - Expression pattern of *mglur6b* in 3 and 5 day old zebrafish

Expression of *mglur6b* transcripts in 3 (A-F) and 5 (G-J) day old zebrafish whole mounts depicted in dorsal (A,B,D,E) and lateral views (C,F,G) as well as in cross sections through the head of 5 day old whole mounts (H-K).

The *mglur6b* riboprobe strongly labels the retinal ganglion cell layer (GCL) and the medial (arrow) and the proximal part (arrowhead) of inner nuclear layer (INL) at 3 dpf (A-C). Weak staining is also found in the olfactory bulb (OB) and the hypothalamus (Hr). Similar staining is found 5 day old larvae (D-G). Cross sections of a 5 day old larvae stained with the *mglur6b* riboprobe (H-J) confirm labeling of few cells in the olfactory bulb (OB), the habenula (Ha), the rostral hypothalamus (Hr) as well as the inner retinal layers (GCL, medial INL: arrow, proximal INL: arrowhead; all in K). Levels of the cross sections H-J are indicated in G. For abbreviations see list. Scale bar in A (applies to A-C) and scale bar in D (applies to D-G) = 50 μm , scale bars in H-K = 20 μm .

Figure 14 - Expression pattern of *mglur7* in 3 and 5 day old zebrafish

In situ hybridization of 3 (A-E) and 5 (F-I) day old zebrafish larvae using the *mglur7* riboprobe.

J-Q depict cross sections through a zebrafish at 5 dpf stained with *mglur7*.

Dorsal (A-C) and lateral views (D,E) of a fish larvae expressing *mglur7* shows staining in broad regions of the CNS from the telencephalon (P) over the diencephalon (Ha, M3, TeO) to the hindbrain (CeP, TG, MO, mn). Additionally, the vagal ganglion (VG) and two layers in the retina (INL, GCL) are stained. Expression of *mglur7* transcripts in the CNS at 5 dpf is even broader compared to 3 dpf as depicted in dorsal (F,G) and lateral (H,I) views of whole mount larvae. In cross sections (J-Q) prominent expression was found in the pallium (P), in diverse diencephalic and midbrain structures such as the optic tectum (TeO) or the hypothalamus (Hr, TLa, DIL), in the nucleus interpeduncularis (Nln), in the cerebellar plate (CeP), in two cranial ganglia (TG, VG), and in the inner part of the INL and the GCL of the retina (Q). Levels of the cross sections J-P are indicated in I. For abbreviations see list. Scale bar in A (applies to A-E) and scale bar in F (applies to F-I) = 50 μm , scale bars in J-Q = 20 μm .

Figure 15 - Expression pattern of *mglur8a* in 3 and 5 day old zebrafish

Expression of *mglur8a* transcripts detected by *in situ* hybridization in 3 day old (A-F) and 5 day old (G-J) fish larvae on whole mounts as well as on cross sections of 5 day old fish heads (K-R).

Whole mount expression of *mglur8a* in dorsal (A-D) and lateral views (E,F) at 3 dpf shows prominent labeling of the preoptic tectum (Po), the cerebellum (CeP), presumably motor neurons (mn) in the hindbrain as well as the retinal ganglion cell layer (GCL). Although the expression seems broader dorsal (G,H) and lateral (I,J) views of *mglur8a* labeled, 5 day old larvae reveal an expression in similar regions of the CNS as 3 day old larvae. Cross sections (K-R) indicate prominent staining in diencephalic areas (e.g. Sd, DT, Po), the medial optic tectum (TeO), the lateral cerebellum (CeP), the torus semicircularis (TS), and the medulla oblongata (MO). Levels of the cross sections K-Q are indicated in J. For abbreviations see list. Scale bar in A (applies to A-F) and scale bar in G (applies to F-J) = 50 μm , scale bars in K-R = 20 μm .

Figure 16 - Expression pattern of *mglur8b* in 3 and 5 day old zebrafish

Expression of *mglur8b* transcripts in 3 (A-G) and 5 (H-K) day old zebrafish whole mounts depicted in dorsal (A-D,H,I) and lateral views (E-G,J,K) as well as in cross sections through the head of 5 day old whole mounts (L-S).

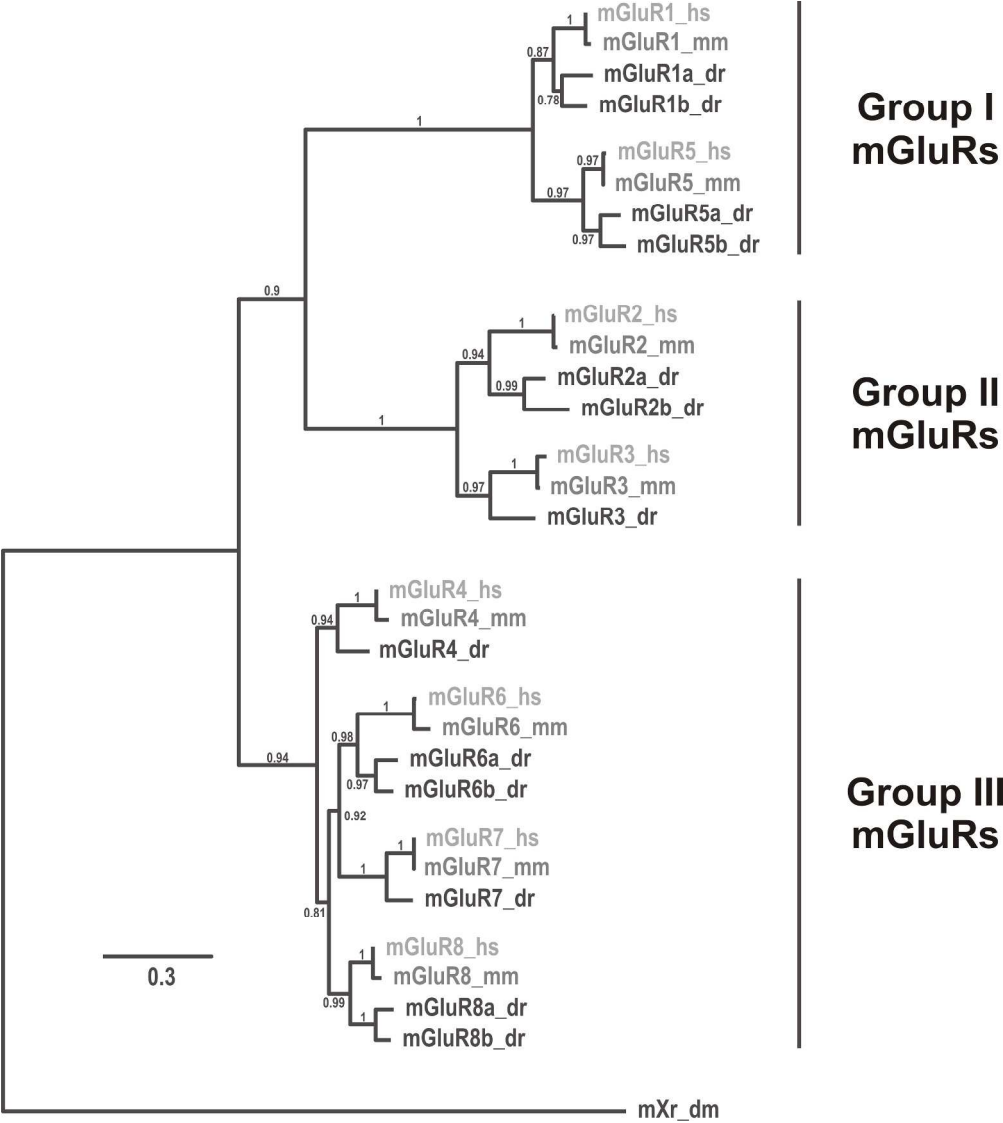
At 3 dpf *mglur8b* is detected in broad regions of the CNS (A-G). Most prominent labeling is found in the olfactory bulb (OB), the optic tectum (TeO), the cerebellum (CeP), in motor neurons (mn) as well as in the retina (GCL, INL). The 5 day old whole mounts (H-K) reveal similar *mglur8b* expression. Cross sections (L-S) confirm the broad expression of *mglur8b* in diencephalic and midbrain (e.g. M3, TeO, DT, PT) as well as hindbrain regions (CeP, TS, MO) of 5 day old fish. In addition, these sections reveal *mglur8b* staining in two cranial ganglia (OG, PLLG). Levels of the cross sections L-R are indicated in K. For abbreviations see list. Scale bar in A (applies to A-G) and scale bar in H (applies to H-K) = 50 μ m, scale bars in L-S = 20 μ m.

Tables

Table 1 - Primer sites used for the generation of sense and antisense RNA probes.

Table 2 - Overview of *mglur* RNA expression in the larval zebrafish CNS. For group I *mglurs* expression in adult tissue is included as well. See list for abbreviations.

For Peer Review



142x159mm (600 x 600 DPI)

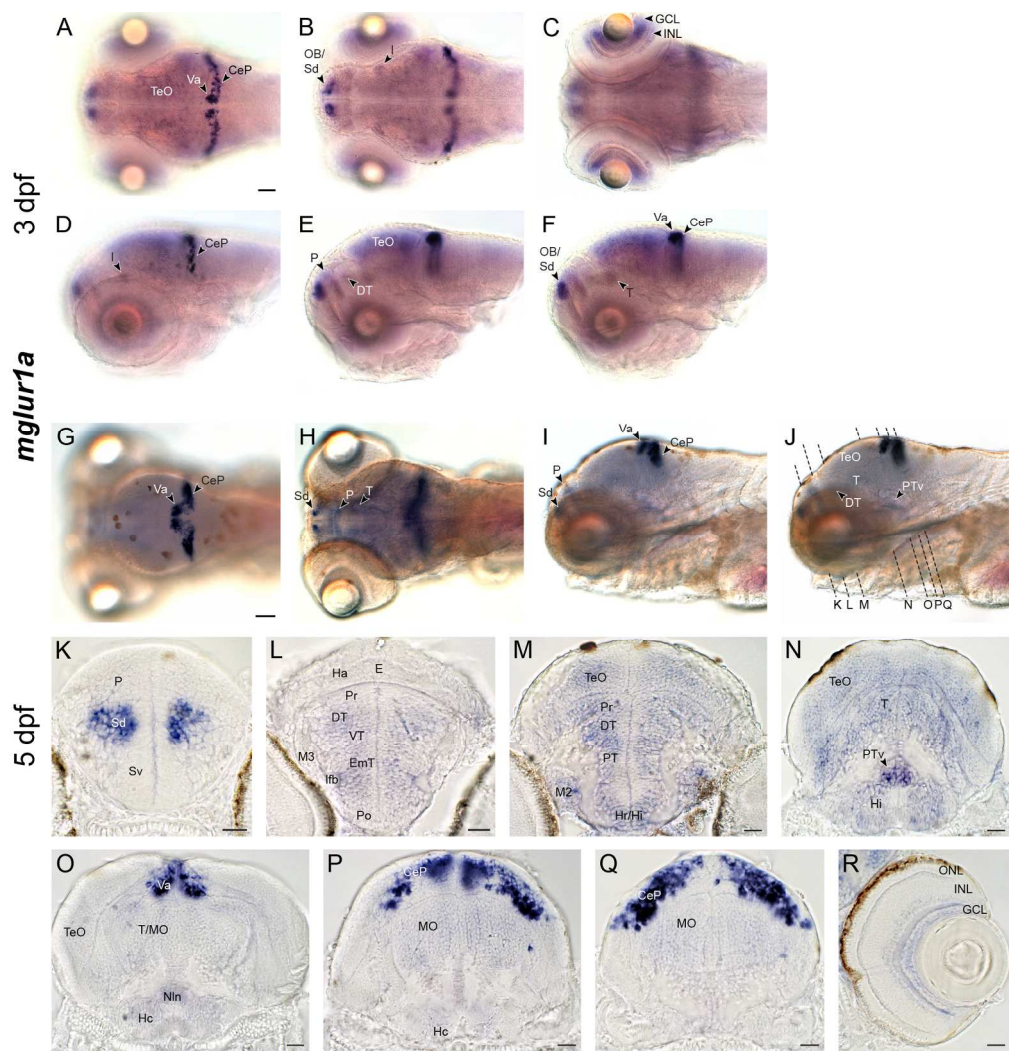


Figure 2
175x182mm (300 x 300 DPI)

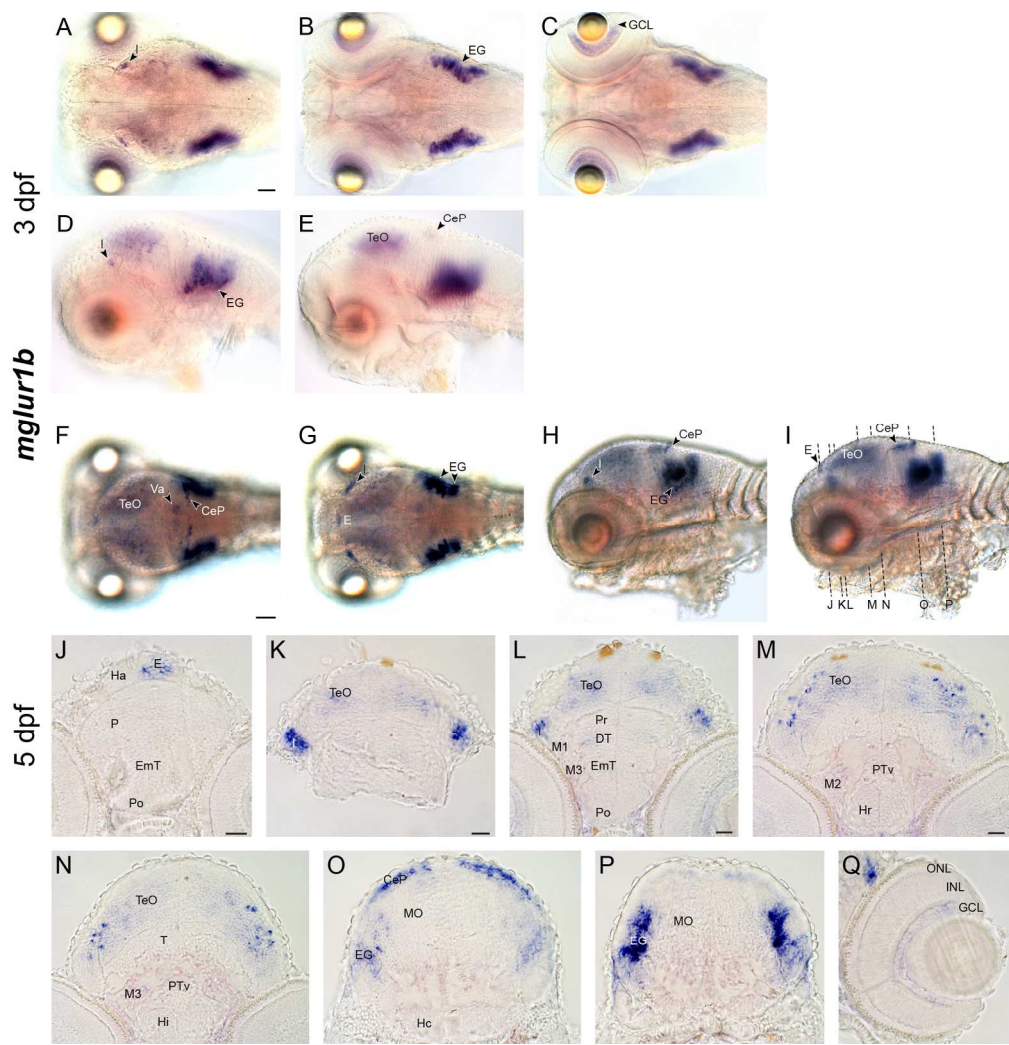
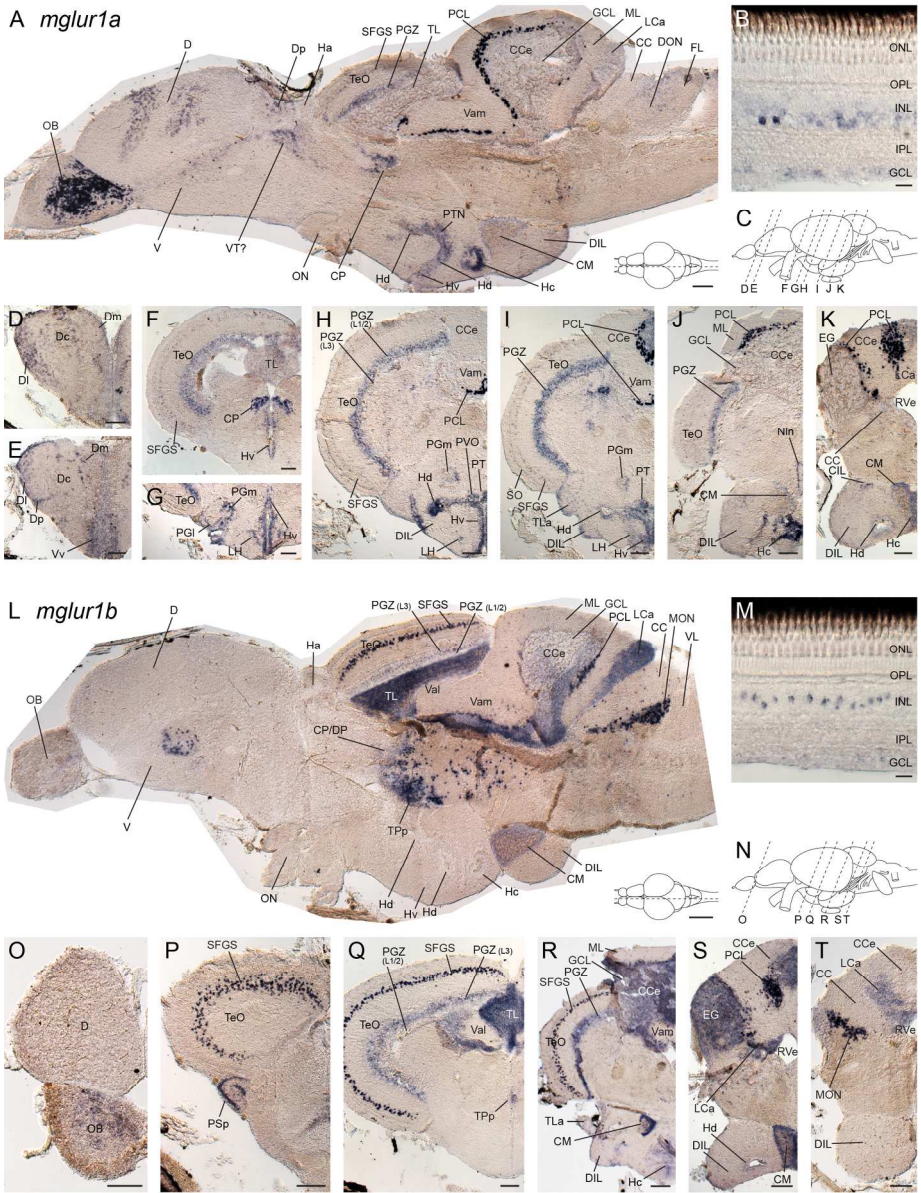


Figure 3
175x180mm (300 x 300 DPI)



172x226mm (300 x 300 DPI)

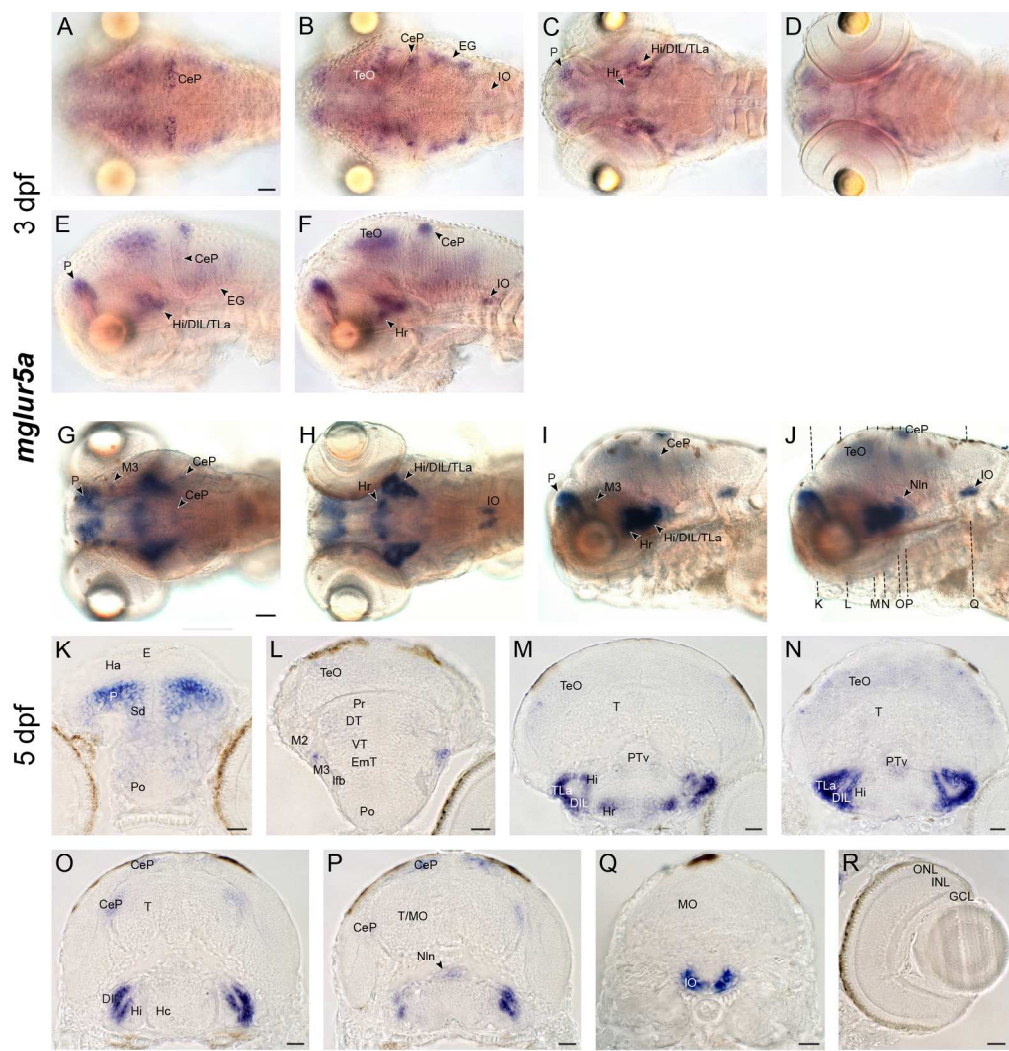


Figure 5
175x184mm (300 x 300 DPI)

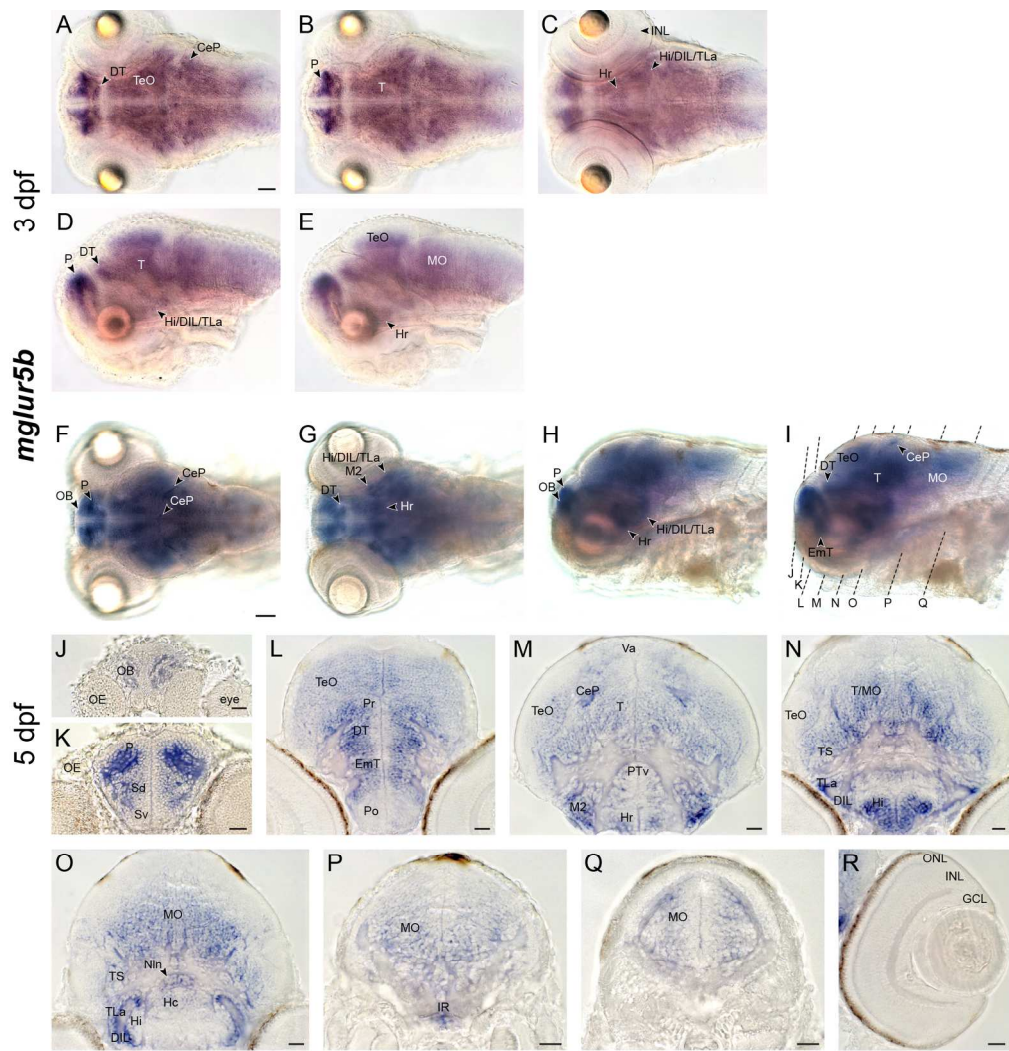


Figure 6
175x184mm (300 x 300 DPI)

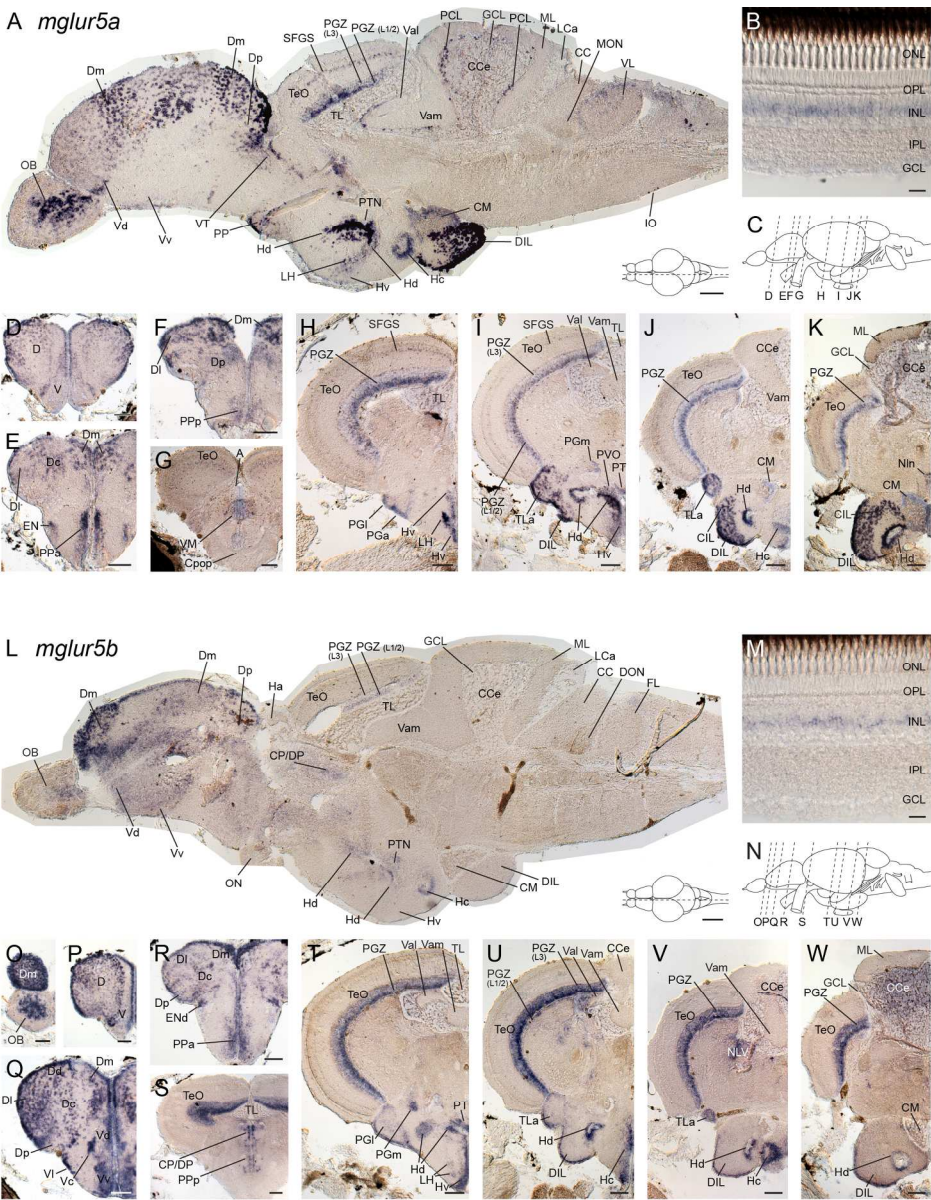


Figure 7
174x222mm (300 x 300 DPI)

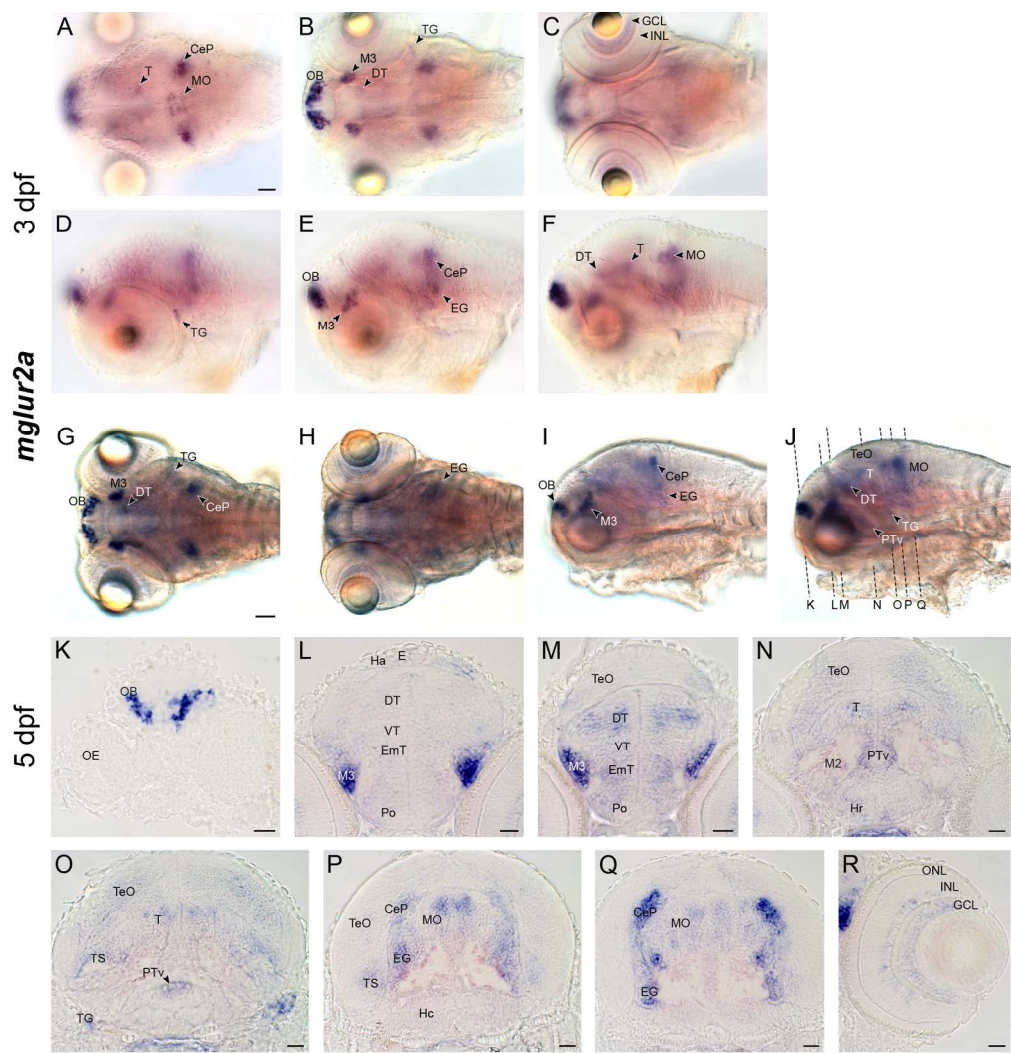


Figure 8
174x182mm (300 x 300 DPI)

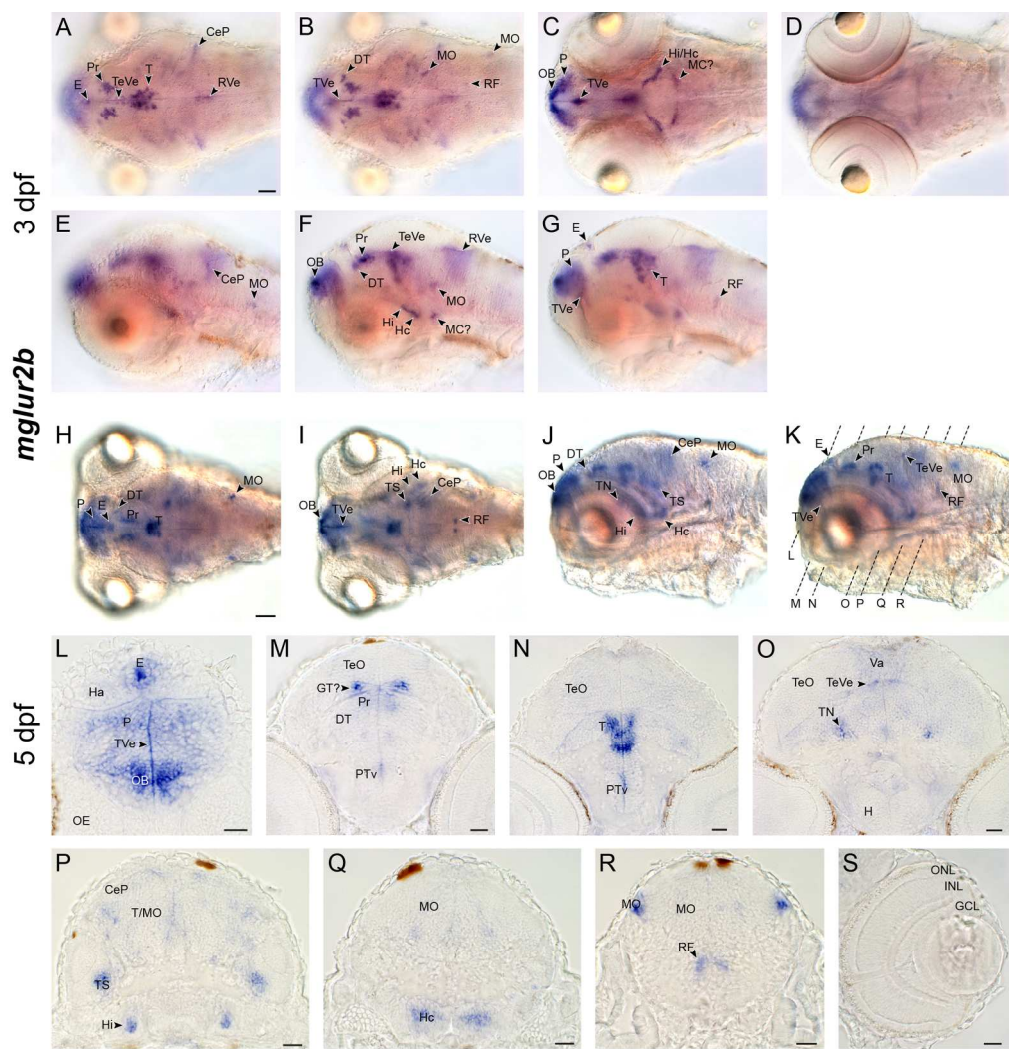


Figure 9
175x182mm (300 x 300 DPI)

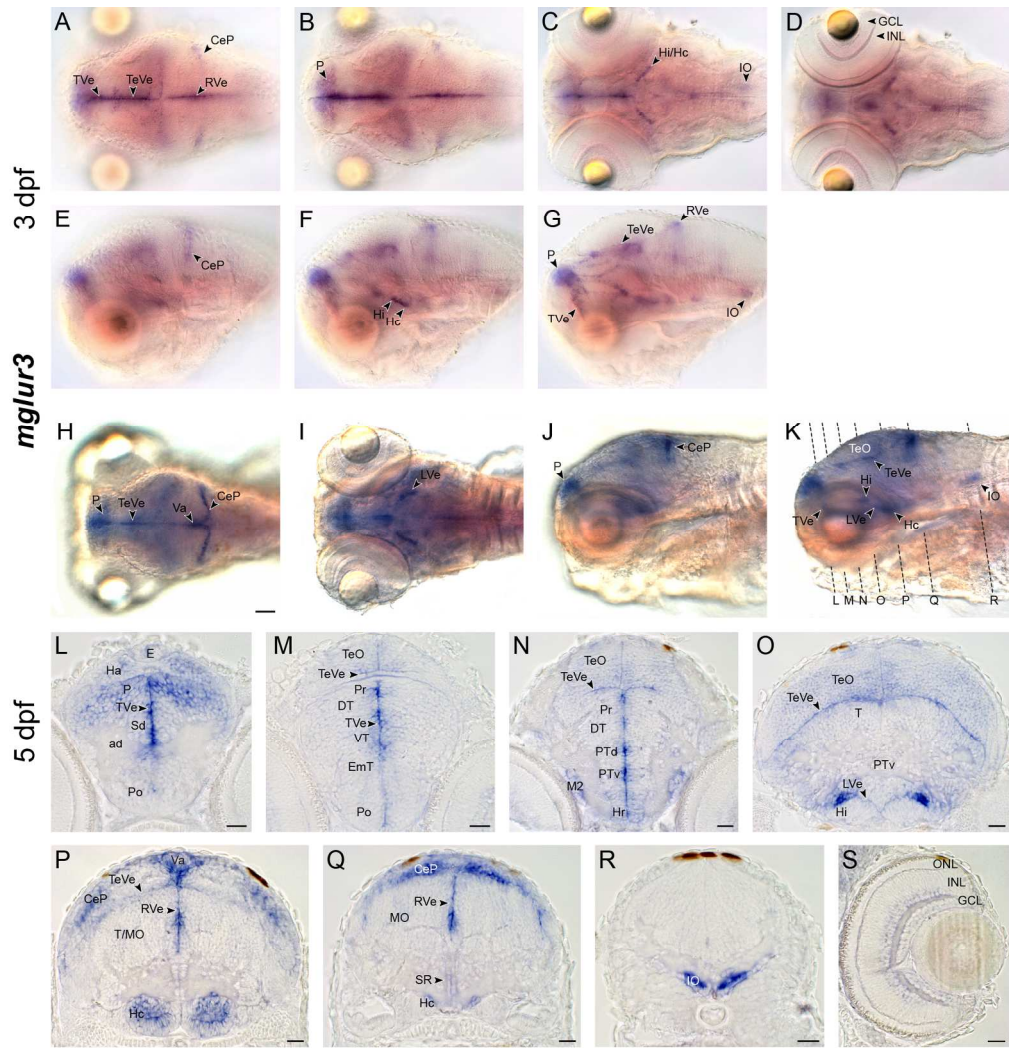


Figure 10
174x182mm (300 x 300 DPI)

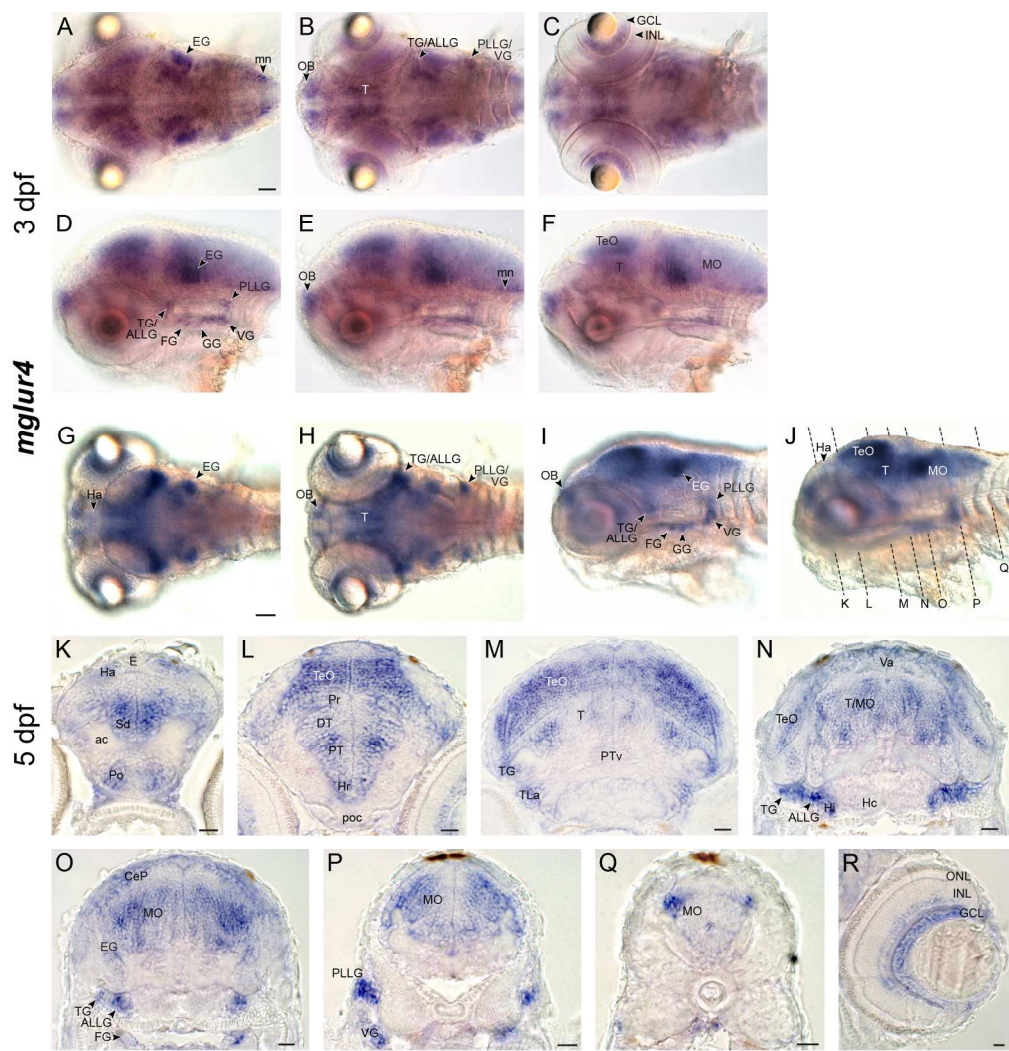


Figure 11
175x182mm (300 x 300 DPI)

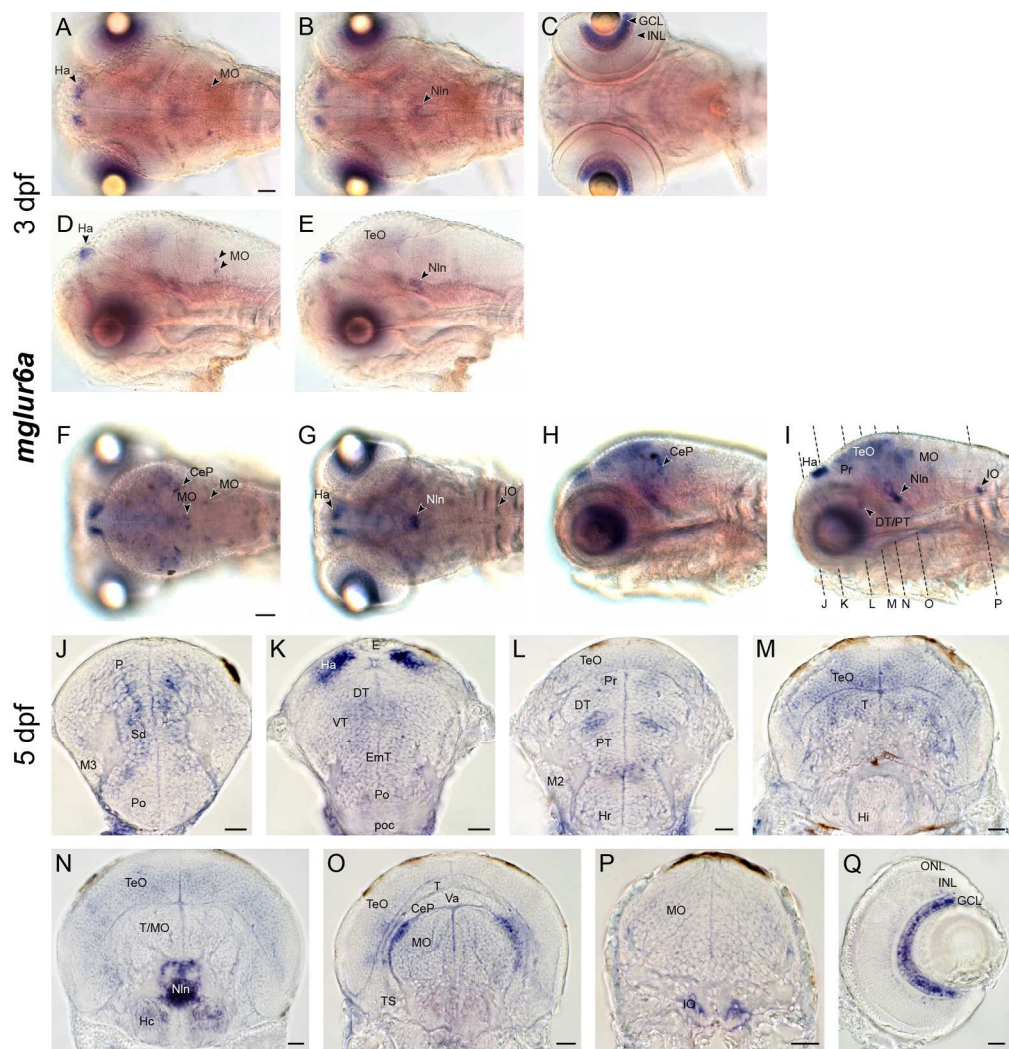


Figure 12
175x182mm (300 x 300 DPI)

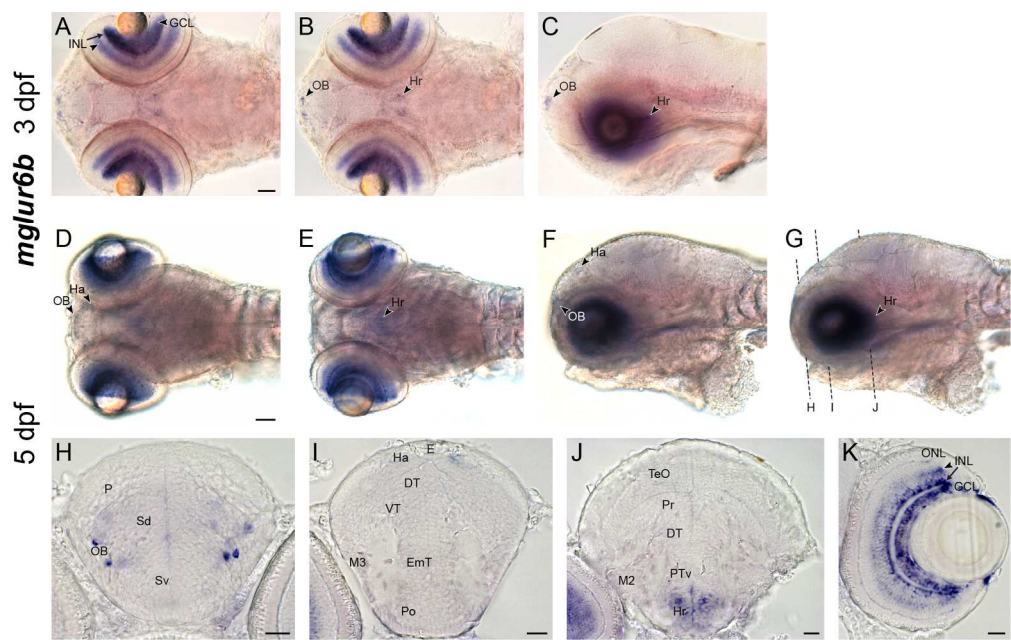


Figure 13
175x110mm (300 x 300 DPI)

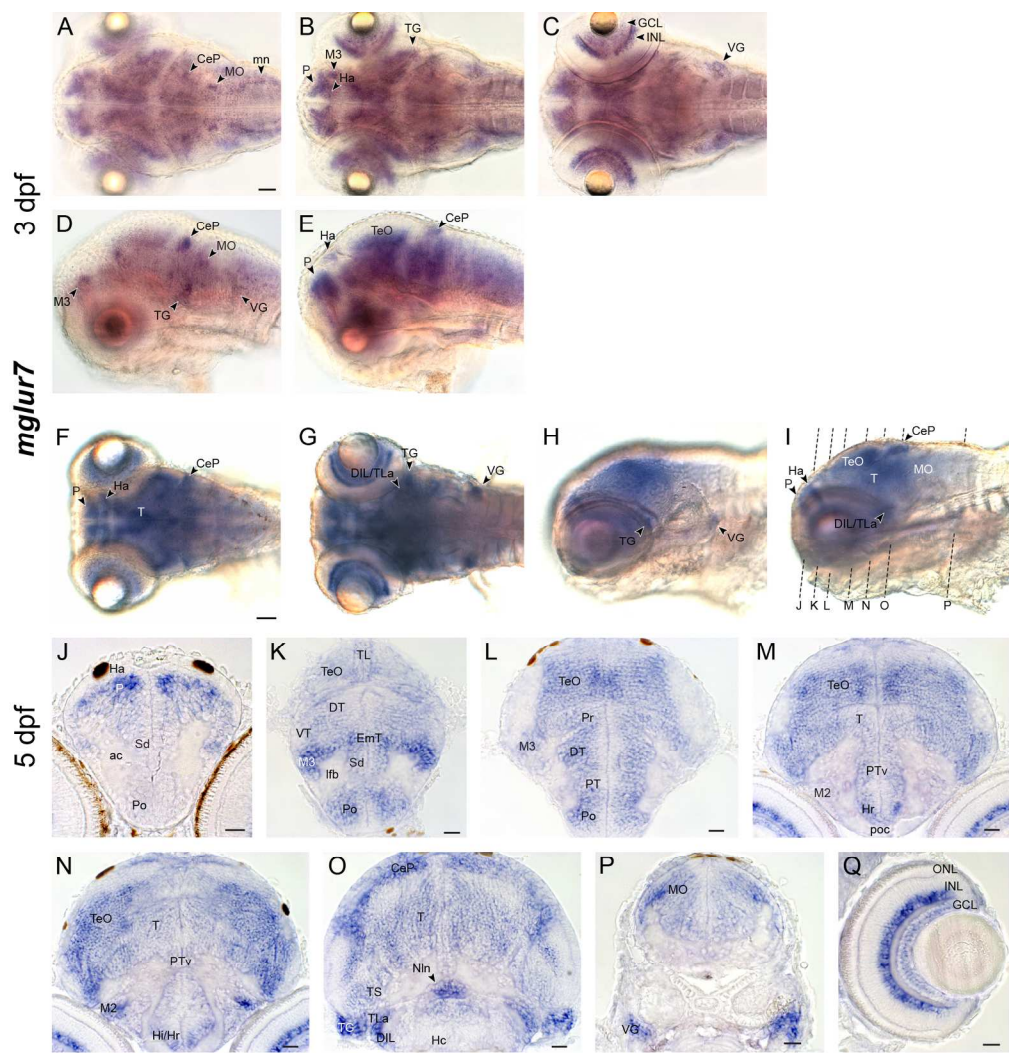


Figure 14
175x184mm (300 x 300 DPI)

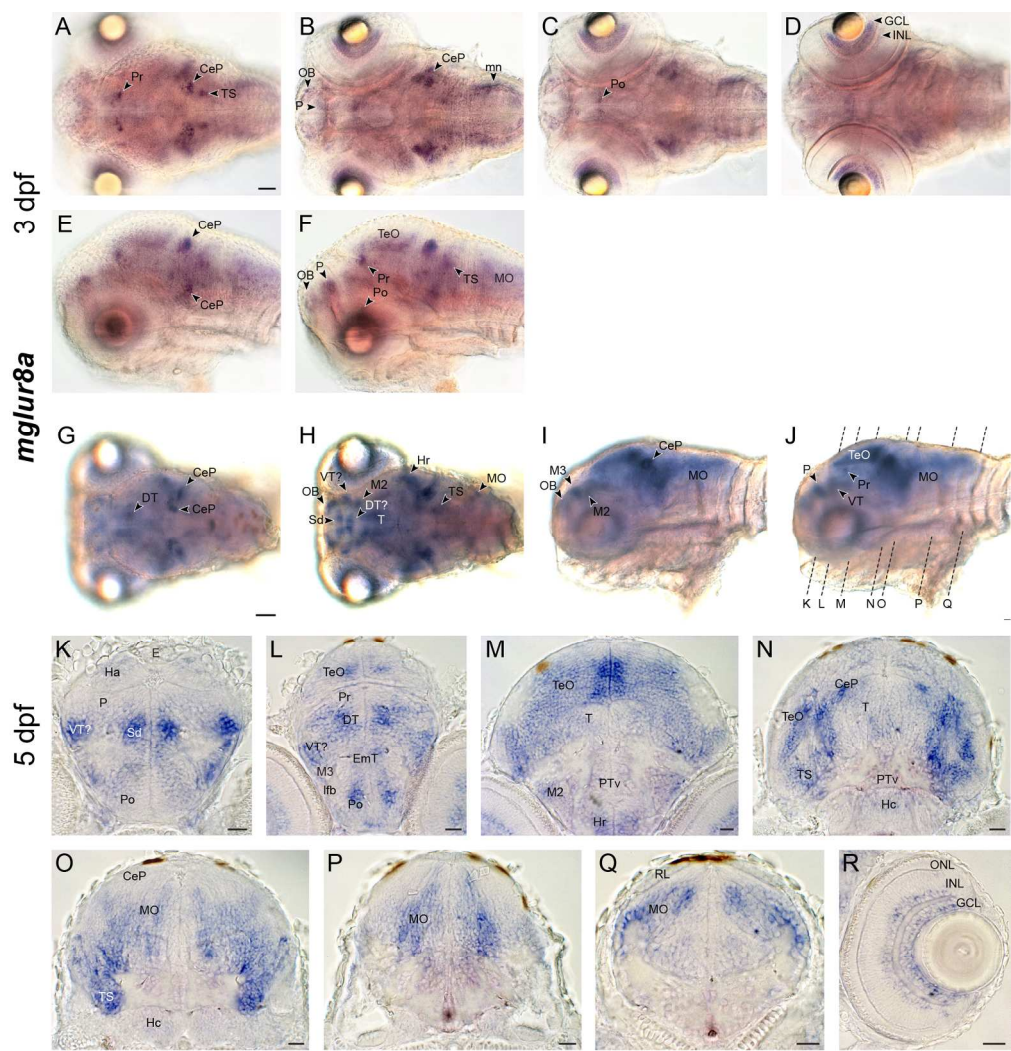


Figure 15
175x183mm (300 x 300 DPI)

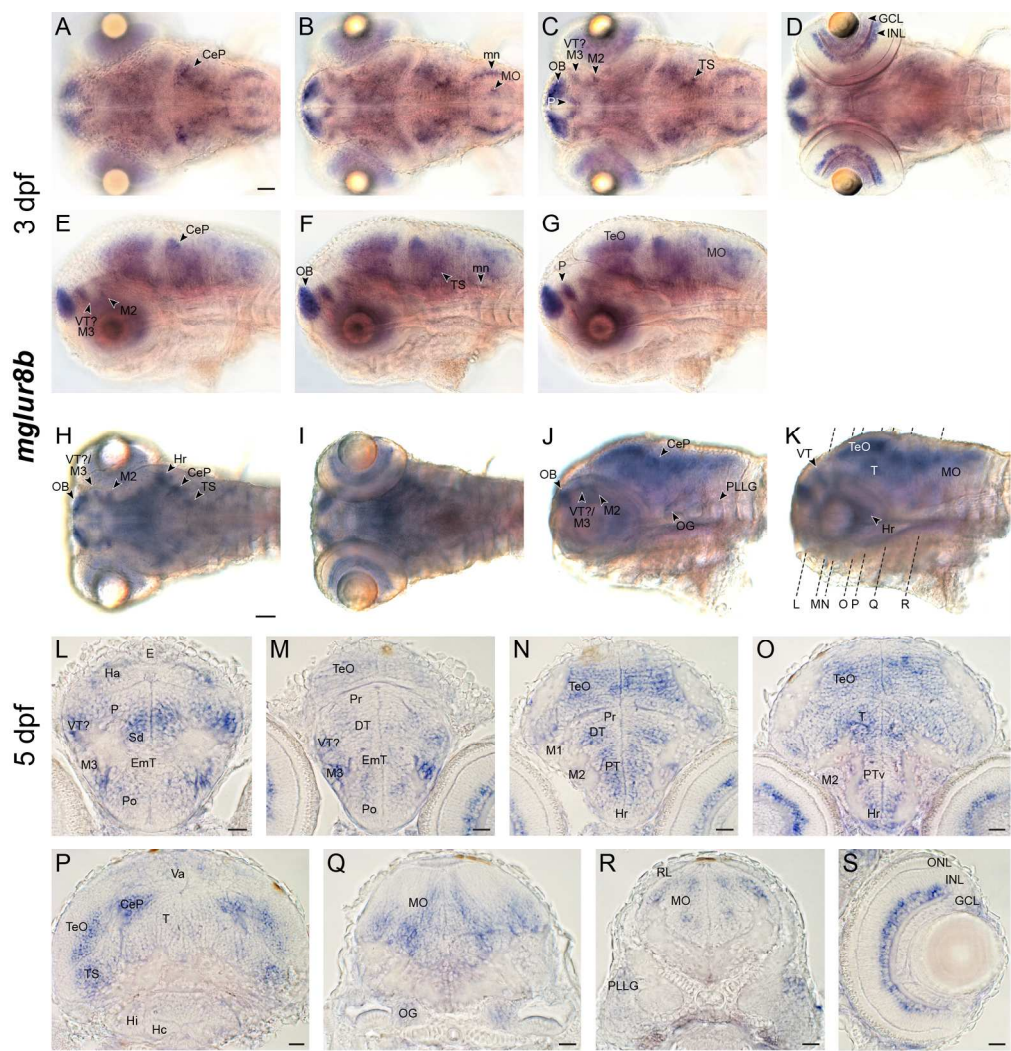


Figure 16
175x182mm (300 x 300 DPI)

Gene	Primer	5' to 3'
<i>mglur1a</i>	1a_dr_1475s	AGGAGATAGGCCGCTTTG
<i>mglur1a</i>	1a_dr_3479as	AGTGATTGATTAATAAACAGCG
<i>mglug1b</i>	1b_dr_1497s	GTGGCATGAAGGCATTTTTG
<i>mglug1b</i>	1b_dr_3501as	ACAAAAAGCTGCCGTAAATC
<i>mglur2a</i>	2a_dr_-0002s	CCATGGCTCAGAGGTCAG
<i>mglur2a</i>	2a_dr_2689as	GGCAAACAAACAGGAATCC
<i>mglur2b</i>	2b_dr_0141	GCACGAGAAGGGAAAAGG
<i>mglur2b</i>	2b_dr_2662	GTGATGACGGGATATAAAATTG
<i>mglur3</i>	3a_dr_-0018s	CTCTTCTGTCTTAAACGATG
<i>mglur3</i>	3a_dr_1652as	CAGGGCATGCAGGTAAAC
<i>mglur4</i>	4a_dr_-0001s	CATGGGGAAAATGATTGG
<i>mglur4</i>	4a_dr_1698as	GTTAGGTCTCAAATCGAAGC
<i>mglur5a</i>	5a_dr_0670s	CACACTGAAGGGAATTATGG
<i>mglur5a</i>	5a_dr_1614s	GACCTGCACTCCATGTAAAG
<i>mglur5a</i>	5a_dr_1931as	TTGGCAATGAGGCAGAAG
<i>mglur5a</i>	5a_dr_3581as	GACATTTACAGCAGGATTTTG
<i>mglur5b</i>	5b_dr_-0001s	ATGGTCATTTTGTGTTCTCTC
<i>mglur5b</i>	5b_dr_3622as	AACACCGCTTAGAATTTTCC
<i>mglur6a</i>	6a_dr_-0003s	AGGATGTTTCGAGTTTTAAGC
<i>mglur6a</i>	6a_dr_1345s	CGCGCTGTCAACTTTAATG
<i>mglur6a</i>	6a_dr_1657as	CGTCTGCTTGGAAGTATAG
<i>mglur6a</i>	6a_dr_2424as	TGCTGTGCCAAAGAAAATG
<i>mglur6b</i>	6b_dr_-0010s	GCCAGCAACTATGACATCAC
<i>mglur6b</i>	6b_dr_1675as	CATCACAGAGCTCACAATGC
<i>mglur7</i>	7a_dr_-0006s	GAGACCATGGCTTGCTTTC
<i>mglur7</i>	7a_dr_1745as	GCCAGGAAGACTGAAATGAC
<i>mglur8a</i>	8a_dr_1300s	AGCAACATCAATGGGAAAG
<i>mglur8a</i>	8a_dr_2733as	TCCTCATATGGCGTGATTG
<i>mglur8b</i>	8b_dr_-0003s	ACCATGTTGAGTGCCTTTG
<i>mglur8b</i>	8b_dr_2785as	AGGATGTTTCGCTCATATGG

region gene			Telencephalon			Diencephalon/Midbrain																Hindbrain							Retina				
			OB	5d: P ad: D	other regions	Ha	E	Eminentia Thalami		Thalamus		Posterior Tuberculum			TeO	Hypothalamus						T	other regions	Cerebellum				Nln	MO	other regions	INL		GCL
								EmT	M3	DT	VT	PTd	PTv	M2		5d: Hr	5d: Hi ad:		Hc	TLa	DIL/ CIL			Va	CCe/ CeP	LCa	EG				prox i-mal	med i-al	
																	ad: PT																
group I	mglur1a	5d	-	+	Sd (++)	-	-	+/-	-	+	-	+/-	++	+	+/-	+	-	-	-	+/-	-	+	++	/	-	+	-	-	+	-	+		
		ad	++	+	V (+)	-	-	-	-	-	+	++	PVO (++)	-	+	++	++	+	++	+	+	-	PGm/l (+) CM (+) CP (++)	-	+/- PCL (++)	+/-	-	+	-	DON (+/-)	++	-	+
	mglur1b	5d	-	-	-	-	++	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	++	/	++	-	-	-	-	-	-	+	
		ad	++	-	Vv (+)	-	-	-	-	-	-	TPp (++)	-	++	-	+/-	-	+	-	-	-	-	PGI (++) CP/DP (+) CM (++)	-	++ PCL (++)	++	++	-	-	MON (++)	++	-	+
	mglur5a	5d	-	++	-	-	-	-	-	-	-	-	+/-	-	+	++	++	-	++	++	-	-	-	+	/	-	+	-	IO (++)	-	-	-	
		ad	++	++	V (+) EN (+)	-	-	-	-	-	-	+	PVO (+) TPp (+)	-	+	+	++	++	+	++	++	-	PP (+) PGaII/m (+)	-	PCL (+/-)	-	-	+	-	IO (++)	-	+	-
	mglur5b	5d	++	++	-	-	-	++	-	++	++	-	-	++	+	+	++	-	+	+	+	-	-	+	/	-	+	+	IR (+)	-	-*	-	
		ad	++	++	V (++) EN (+)	-	-	-	-	-	-	++	-	++	++	++	++	++	+	+	-	CP/DP (+) PGaII/m (++)	+/-	+/-	-	-	-	-	NLV (++)	-	+	-	
	group II	mglur2a	5d	++	-	-	+/-	-	+/-	++	+	-	-	+	-	+/-	-	-	-	-	+	-	-	++	/	+	-	+	TG (++) TS (+)	+	-	+	
		mglur2b	5d	++	+	TVe (+)	-	++	-	+	+	-	-	+	-	+	-	++	++	-	-	++	Pr (++) TeVe (+)	+/-	+	/	-	-	+	RF (+) TS (++) MC? (++)*	-	-	-
		mglur3	5d	-	++	TVe (++)	+	-	-	-	-	-	+	+	+	-	-	++	++	-	-	-	TeVe (++) LVe (+) SR (+)	++	++	/	-	-	-	RVe (++) IO (++)	+	-	+
	group III	mglur4	5d	+	-	Sd (++) Po (+)	+	-	-	-	-	-	+	+	-	++	+/-	++	-	+	-	++	-	-	+	/	+	-	++	cranial ganglia (++)	+	-	++
		mglur6a	5d	-	-	Sd (+/-)	++	-	-	-	+/-	+/-	+/-	+/-	-	+	-	-	+	-	-	+	-	-	+	/	-	++	-	IO (+)	+	-	++
		mglur6b	5d	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	/	-	-	-	-	++	+	++	
		mglur7	5d	-	++	Po (+)	+	-	+	++	+	-	+/-	+/-	++	++	+	+	-	+	++	++	-	-	++	/	-	++	+	TG (++) VG (++)	++	-	+
		mglur8a	5d	+	-	Sd (++) Po (+)	-	-	-	+	+	+	-	-	+	++	+	-	-	-	-	-	-	++	/	-	-	++	TS (++)	+	-	+	
		mglur8b	5d	+	-	Sd (+)	+	-	-	+	+/-	+	+	+	+/-	++	+	-	-	-	-	+	-	-	++	/	-	-	+	OG (+) PLLG (+) TS (+)	++	-	-

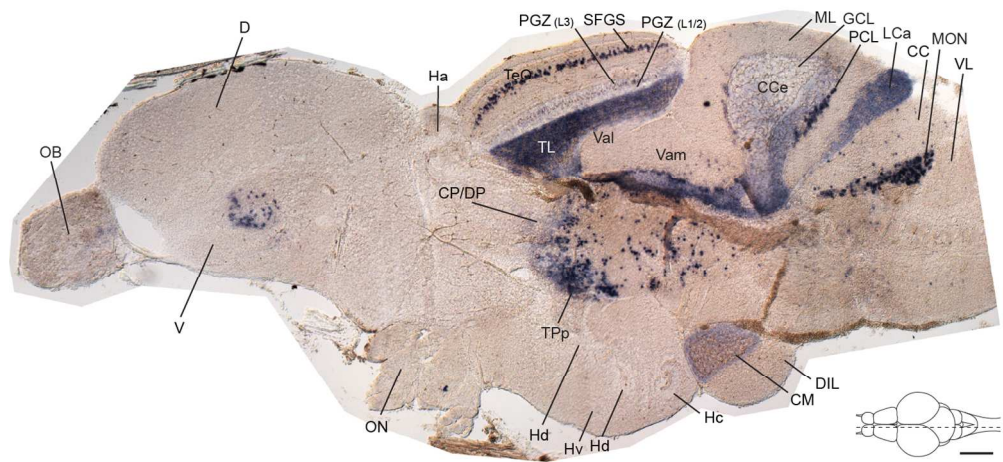
- no expression; +/- very weak expression; + medium expression; ++ strong expression

* only at 3 dpf

/ only relevant in adult tissue

We describe the cloning, phylogenetic analysis and mRNA distribution of zebrafish metabotropic glutamate receptor genes (mGluRs). The 13 zebrafish *mglur* genes display well defined expression domains in the central nervous system, most prominently in the olfactory bulb, optic tectum, hypothalamus, cerebellum, and retina. Expression of paralogous genes shows both overlapping and complementary expression, suggesting sub- and/or neofunctionalization. Expression of group I *mglurs* revealed comparable patterns in larval and adult brains, suggesting similar functions at different developmental stages.

For Peer Review



135x67mm (300 x 300 DPI)

Peer Review